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Obstetric and Gynaecological Aspects of HIV infection in Finland

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I Lehtovirta P, Skogberg K, Salo E, Ämmälä P, Ristola M, Suni J, Paavonen J and Heikinheimo O. Pregnancy outcome among HIV-infected women in the Helsinki metropolitan area. Acta Obstetricia et Gynecologica Scandinavica 2005; 84 (10): 945-950.

II Heikinheimo O, Lehtovirta P, Suni J and Paavonen J. The levonorgestrel-releasing intrauterine system (LNG-IUS) in HIV-infected women – effects on bleeding patterns, ovarian function and genital shedding of HIV. Human Reproduction 2006; 21:2857-2861.

III Lehtovirta P, Paavonen J and Heikinheimo O. Experience with the levonorgestrel-releasing intrauterine system (LNG-IUS) among HIV-infected women. Contraception 2007; 75: 37-39.

IV Lehtovirta P, Finne P, Nieminen P, Skogberg K, Savonius H, Paavonen J and Heikinheimo O. Prevalence and risk factors of squamous intraepithelial lesions of the cervix among HIV-infected women – a long-term follow-up study in a low prevalence population. International Journal of Sexually Transmitted Diseases and AIDS 2006; 17:831-834.

V Lehtovirta P, Paavonen J and Heikinheimo O. Risk factors, diagnosis and prognosis of cervical intraepithelial neoplasia among HIV infected women. International Journal of Sexually Transmitted Diseases and AIDS accepted 7/07.

ABBREVIATIONS

AGUS	atypical glandular cells of uncertain significance
AIDS	acquired immunodeficiency syndrome
ALIVE	AIDS link to intravenous drug experience
ARV	antiretroviral
ASCUS	atypical squamous cells of uncertain significance
BV	bacterial vaginosis
CIN	cervical intraepithelial neoplasia
CI	confidence interval
CS	Caesarean section
Cu-IUD	copper-releasing intrauterine device
DITRAME	Diminution de la Transmission Mere-Enfant
ELISA	enzyme-linked immunosorbent assay
E2	oestradiol
HAART	highly active antiretroviral therapy
Hb	haemoglobin
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HIVNET	HIV Network for Prevention Trials
HPS	high pure system
HPV	human papilloma virus
HSIL	high-grade squamous intraepithelial lesion
ICC	invasive cervical cancer
IVF	*in vitro* fertilization
LEEP	loop electrosurgical excision procedure
LNG	levonorgestrel
LNG-IUS	levonorgestrel-releasing intrauterine system
LSIL	low-grade squamous intraepithelial lesion
NNRTI	non-nucleoside analogue reverse transcriptase inhibitor
NRTI	nucleoside analogue reverse transcriptase inhibitor
NS	not significant
NVP	nevirapine
PACTG	Pediatric AIDS Clinical Trials Group
Pap	Papanicolaou
PETRA	Perinatal Transmission Trial
PI	HIV-1-specific protease inhibitor
PID	pelvic inflammatory disease
RNA	ribonucleic acid
SD	standard deviation
STD	sexually transmitted disease
US	ultrasonography
WHO	World Health Organization
WIHS	Women's Interagency HIV Study
WITS	Women and Infant's Transmission Study
ZDV	zidovudine
3TC	lamivudine

ABSTRACT

The prevalence of human immunodeficiency virus (HIV) infection has increased among the female population worldwide, and to a lesser extent in Finland. The problems associated with HIV infection among women especially concern the effects on pregnancy, vertical transmission, contraception and prevention of HIV horizontal transmission, and the increased risk of cervical pre-malignant lesions and cancer of the cervix.

We examined the outcome of pregnancies among HIV-infected women during 1993–2003 in Helsinki, use of the levonorgestrel-releasing intrauterine system (LNG-IUS) among HIV-infected women and the prevalence and risk factors of cytological and histologically proven cervical lesions in this population.
Between 1993 and 2003 a total of 45 HIV-infected women delivered 52 singleton infants. The annual prevalence of HIV infection among women delivering in the hospital district of Helsinki and Uusimaa increased from 0.6/10 000 to 4.8/10 000 between 1993 and 2002. HIV infection was diagnosed during pregnancy in 18/45 (40%) of the mothers. Seventeen of the mothers received antiretroviral (ARV) medication prior to pregnancy and in 34 (66%) cases, the medication was started during pregnancy. A good virological response (i.e. HIV RNA load <1000/mL during the last trimester) to ARV medication was achieved in 36/40 (90%) of the patients in whom HI viral load measurements were performed; in 24/40 (60%) of the patients HIV RNA load was below the detection limit of the assays. Of the infants, 92% were born at term, and their mean (±SD) birth weight was 3350±395 g. The Caesarean section (CS) rate was low, 25%. All newborns received ARV medication and none of the infants born to mothers with pre-delivery diagnosis of maternal HIV infection were infected.

The safety and advantages of the LNG-IUS were studied prospectively (14 months) among 12 HIV-infected women and retrospectively (mean duration of follow-up 45 months) among six HIV-infected women. The LNG-IUS was well tolerated and no cases of PID or pregnancy were noted. Menstrual bleeding was reduced significantly during use of the LNG-IUS; this was associated with a slight increase in haemoglobin levels. In a prospective study, serum oestradiol (E2) concentrations remained in the follicular range in all subjects. Among subjects using ARV medication, the proportion of cervicovaginal lavage specimens with detectable HIV RNA was 10% both before and after insertion of an LNG-IUS. No Pap smear changes were observed and the level of CD4 lymphocytes remained stable throughout the follow-up period.

Data on 108 systematically followed HIV-infected women during 1989–2003 and on 153 HIV-infected women during 1989–2006 were collected for analysis of cytological Pap smear abnormalities and for analysis of histologically verified dysplasia, respectively. The mean prevalences of low-grade squamous intrapithelial lesions (LSIL) and high-grade SIL (HSIL) were high: 15% and 5%, respectively. A reduced CD4 lymphocyte count was associated with an increased prevalence of SIL, whereas duration of HIV infection, use of ARV medication and HI viral load were not. The cumulative risk of any type of SIL was 17% after one year and 48% after five years among patients with initially normal Pap smears. The risk of developing SIL was associated with young age and a high initial HI viral load. However, CD4 level, ARV medication, HCV co-infection and smoking were not associated with the development of SIL. During the follow-up 51 subjects (33%) displayed cervical intraepithelial neoplasia (CIN), (16% CIN1 and 18% CIN 2-3). Only one case of cancer of the uterine cervix was detected. Pap smears were reliable in screening for CIN; 75% of CIN patients showed HSIL or LSIL in Pap smears taken at the time of dysplasia. The incidence of CIN showed a decreasing tendency from 12.7 to 3.5 (per 100 subjects) between 2000 and 2005 (p=0.07). The risk of CIN was not associated with decreased levels of CD4 lymphocytes, duration of HIV infection, use of ARV medication or plasma HI viral load. However, both nulliparity (p<0.01) and BV (p<0.04) emerged as significant risk factors of CIN.
Cervical intraepithelial neoplasia was treated by means of LEEP (n=34). The recurrence rate was low; 16% of the subjects showed recurrence during the follow-up period. The nadir of the CD4 lymphocyte count was lower (p=0.04) and the HI viral load higher (p=0.03) among subjects with CIN recurrence. Duration of HIV infection, use of ARV medication, and positive margins were indistinguishable among subjects with and without CIN recurrence.

In conclusion, a combination of universal antenatal screening and multidisciplinary management allows individualized treatment and prevents vertical transmission of HIV. Use of the LNG-IUS is safe among HIV-infected women and cervicovaginal shedding of HIV RNA is not affected by use of the LNG-IUS. The risk of cervical pre-malignant lesions is high among HIV-infected women despite systematic follow-up.

INTRODUCTION

Since its recognition in 1981 among men diagnosed with *Pneumocystis carinii pneumonia* (Centers for Disease Control, 1981), human immunodeficiency virus infection has become a pandemic. Global estimates are that 39.5 million (34.1–47.1 million) people live with HIV/AIDS worldwide, while about 25 million have already died (UNAIDS, 2006). According to the WHO, HIV infection has become the fourth leading cause of death and among infectious pathogens HIV causes more deaths (2.9 million in 2006) than any other single agent (UNAIDS 2006, www.who.org). In the early years of the epidemic, HIV was perceived mainly as a problem of homosexuals. Today the HIV pandemic is rapidly becoming a burden of the female population; of the 37 million HIV-infected adults, 18 million are women and 500 000 HIV-infected children are born annually (UNAIDS 2006, www.who.org).

The prevalence of HIV infection among women has remained low in Finland, but has increased recently: 60 women were diagnosed with HIV in 2006 compared with 20–40 per year between 1996 and 2005 (www.ktl.fi).

Although the effects of recent developments, namely introduction of highly active antiretroviral therapy (HAART) in 1995 and various prevention programmes have been significant, the disease profile and outcome differ greatly in resource-poor versus developed countries. In developing countries the mortality rates are high; total HIV-related deaths in South Africa increased by 79% from 1997 to 2004 (Statistics South Africa, 2006), whereas in the developed world the availability of HAART has resulted in an increased lifespan of HIV-infected patients (Palella *et al*., 1998, Hammer, 2005). HIV infection is now defined as a chronic as opposed to a fatal disease. With improved life expectancy and quality of life among HIV-infected patients in developed countries, reproductive aspects of the infection are becoming increasingly important.

In developing countries the main concern as regards HIV infection among women lies in prevention of vertical transmission. In developed countries more efforts and resources are focused on the prevention of CIN and cancer of the cervix, and on offering effective and safe contraceptive methods. In addition, assisted reproductive techniques are being designed for HIV-infected couples.

Much of the research data on gynaecological aspects of HIV is derived from cross-sectional studies carried out in countries whose health care systems differ from the Finnish system. The Department

of Obstetrics and Gynaecology at the University of Helsinki has offered gynaecological and obstetric care to HIV-infected women since 1993, and these women have been followed up systemically at 6- to 12-month intervals. In May 2007 the number of women enrolled for follow-up was 252.

This study evaluates pregnancy outcome, safety and advantages of the LNG-IUS as well as the prevalence and risk factors of cervical atypia among HIV-infected women in Finland.

REVIEW OF THE LITERATURE

1. HIV INFECTION

1.1 Aspects of HIV

The human immunodeficiency virus is a retrovirus belonging to the group of lentiviruses. The genome size of HIV is modest (less than 10 kb) and it has only a few genes (Simon *et al*., 2006). HIV primarily infects cells in the human immune system such as CD4 lymphocytes, macrophages and dendritic cells. Infected cells move to regional lymphoid tissue, where the HI virus replicates for days to weeks (Kassutto *et al*., 2004). Symptoms of the primary infection include fever, sore throat, skin rash, lymphadenopathy, splenomegaly, myalgia, arthritis and meningitis (Fauci *et al*., 1996). These symptoms are very similar to the symptoms of mononucleosis. The viral load rises exponentially during the primary infection and after that declines to reach a set point at 6 months (Perrin, 1999). The magnitude of the initial viral load set point is prognostic as regards disease progression (Lyles *et al.*, 2000). HIV infection gradually leads to low levels of CD4 lymphocytes and the patient becomes progressively more susceptible to opportunistic infections and malignancies (Table 1). The infection is characterized by a long asymptomatic period (of up to more than 10 years) between primary infection and symptomatic AIDS (Pantaleo *et al*., 1993, Grossman *et al*., 2006) (Figure 1). The AIDS state is defined by one of the opportunistic infections or malignancies listed in Table 1.

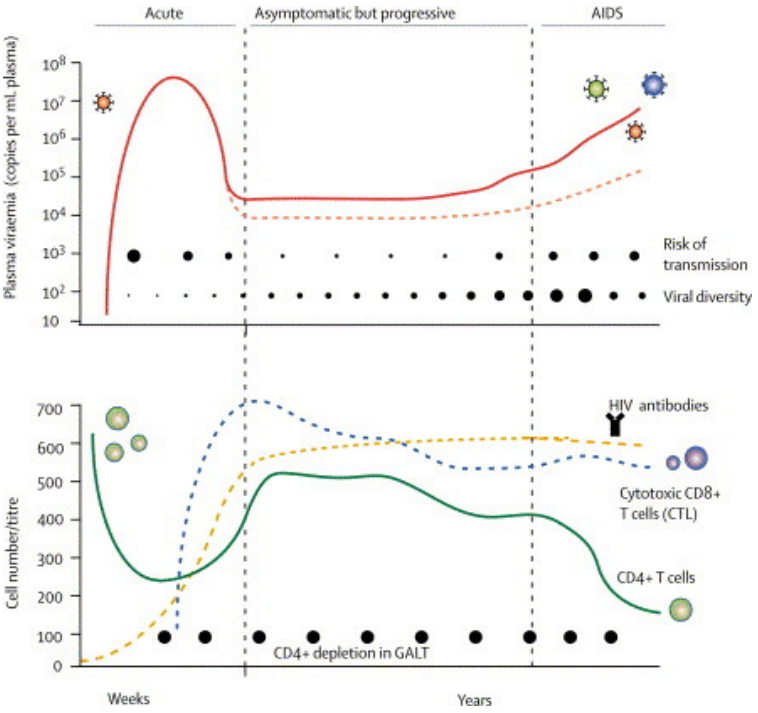


Figure 1. The course of HIV infection defined by the level of viral replication.
Plasma viraemia (top figure), and changes in the CD4+ T-lymphocyte compartments (bottom figure). Primary infection is characterised by high levels of plasma HIV (red line, top), low levels of CD4 cells (green line, bottom), and absence of HIV antibodies (orange line, bottom). Viraemia drops as cytotoxic CD8+ T-lymphocytes (CTL) develop (blue line, bottom) and an individual viral load set point is reached during chronic infection. Viral set points differ greatly among individuals (e.g. red dotted line, top) and predict disease progression. Viral diversity increases throughout the disease (closed circles, top). The risk of transmission is highest in the first weeks when viraemia peaks (closed circles, top). GALT = gut-associated lymphoid tissues. (Simon *et al*., 2006).

Table 1. Twenty opportunistic infections and malignancies that define progression to the AIDS state after HIV infection. (www.aegis.com/topics/definition, assessed on 15th Aug 2007):

1. Candidiasis of the bronchi, trachea, esophagus or lungs
2. Cervical cancer
3. Coccidioidomycosis, disseminated or extrapulmonary
4. Cryptococcosis, extrapulmonary
5. Cytomegalovirus retinitis (with loss of vision)
6. Encephalopathy

7. Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or oesophagitis
8. Histoplasmosis, disseminated or extrapulmonary
9. Isosporiasis, chronic intestinal (greater than 1 month's duration)
10. Kaposi's sarcoma
11. Lymphoma, Burkitt's (or equivalent term)
12. Lymphoma, immunoblastic (or equivalent term)
13. Lymphoma, primary, of brain
14. *Mycobacterium avium* complex or *M. kansasii*, disseminated or extrapulmonary
15. *Mycobacterium tuberculosis*, any site (pulmonary or extrapulmonary)
16. *Pneumocystis carinii pneumonia*
17. Progressive multifocal leukoencephalopathy
18. Salmonella septicaemia, recurrent
19. Toxoplasmosis of the brain
20. Wasting syndrome due to HIV

Before the introduction of ARV medication the median survival time after diagnosis of AIDS was 12 to 18 months, but the use of ARV medication has greatly improved the prognosis of HIV infection (Palella *et al*., 1998, Hammer, 2005). HAART has led to a redefinition of HIV infection from a fatal to a chronic disease, and life expectancy is approaching that of HIV-negative controls. Antiretroviral medication is categorized in four classes (Figure 2):

1. Nucleoside analogue reverse transcriptase inhibitors (NRTIs) (for example ZDV, 3TC, didanoside, stavudine, zalcitabine, abacavir)
2. Non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs) (for example NVP, delavirdine, efavirenz)
3. Protease inhibitors (PIs) (for example indinavir, ritonavir, saquinavir, nelfinavir, lopinavir)
4. Fusion inhibitors (for example enfuvirtidi), entry inhibitors, integrase inhibitors

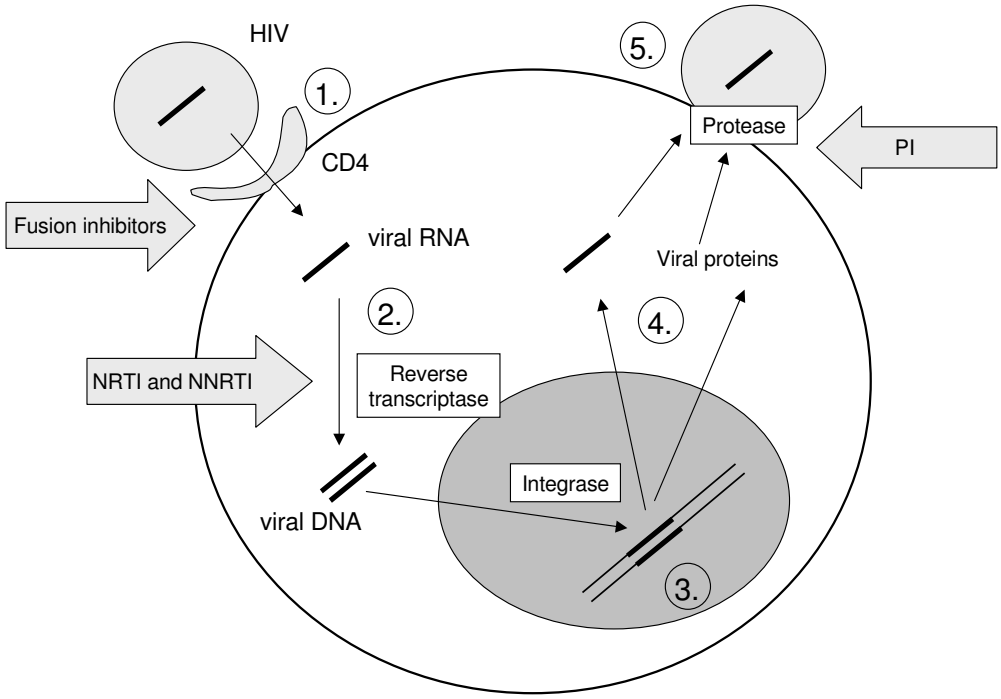


Figure 2. Schematic picture of the life cycle of HIV and the site of action of antiretroviral agents. HIV enters the human host cell by using CD4- and co-receptors (1). The released viral single-stranded RNA is converted into double-stranded DNA by viral reverse transcriptase (2). Double-stranded viral DNA enters the cell nucleus and is incorporated into the cell DNA in a reaction catalyzed by the viral integrase enzyme (3). Viral proteins and RNA are synthesized (4) and new viruses assembled (5). Viral proteins are modified by the viral protease. Fusion inhibitors block viral entry into the cell; NRTIs and NNRTIs inhibit the reverse transcriptase and PIs the protease enzyme. (Sutinen, 2003).

1.2 Epidemiology

The HIV pandemic has undoubtedly become the public health crisis of our time. An estimated 39.5 million people worldwide were living with HIV at the end of 2006 and about 25 million have died because of it (UNAIDS 2006, www.who.org). In 2005 alone, there were 4.2 million new HIV infections and 2.8 million AIDS deaths. Africa remains the global centre of the HIV pandemic; sub-Saharan Africa's HIV epidemic shows no evidence of decline. The epidemics in Eastern Europe and Asia are expanding, especially in Ukraine and in the Russian Federation. Figure 3 shows the numbers of new HIV infections in Europe in 2005.

The prevalence of HIV infection has remained relatively low in Finland. In July 2007 the number of reported HIV-infected individuals was 2191, giving a prevalence of HIV of 4/10 000 (www.ktl.fi). The annual number of new HIV infections (128–144) has been relatively stable during the early 21st century, but in 2006 a steep increase (193) was noticed. In Finland the number of HIV-infected women was 565 in July 2007 and 82% of them resided in the greater Helsinki area (www.ktl.fi). Figure 4 shows the newly diagnosed HIV infections in Finland among women and men. The increasing incidence in the countries neighbouring Finland is alarming. For example, in Estonia the prevalence of HIV infection is 1% in the adult population and the prevalence among prostitutes is as high as 7% (www.eurohiv.org). The prevalence of HIV infection in Finland and in neighbouring countries is shown in Table 2.

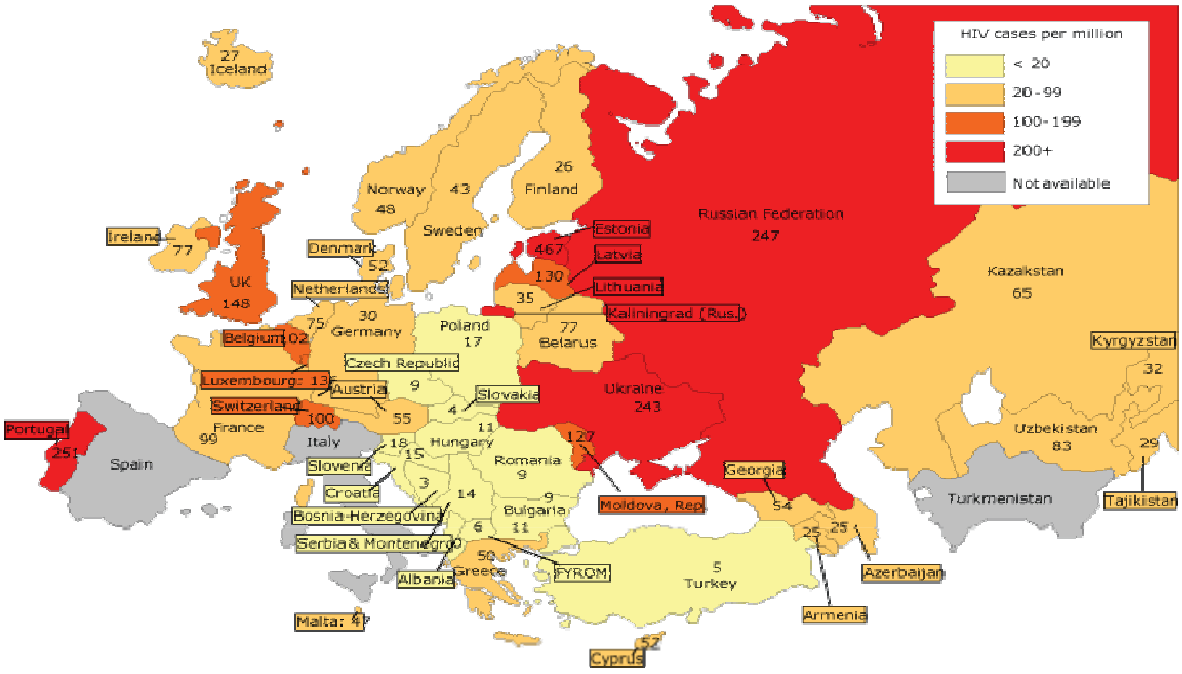


Figure 3. Newly diagnosed HIV infections: cases reported in 2005 per million population; WHO European Region (www.eurohiv.org). Assessed on 31st Aug 2007.

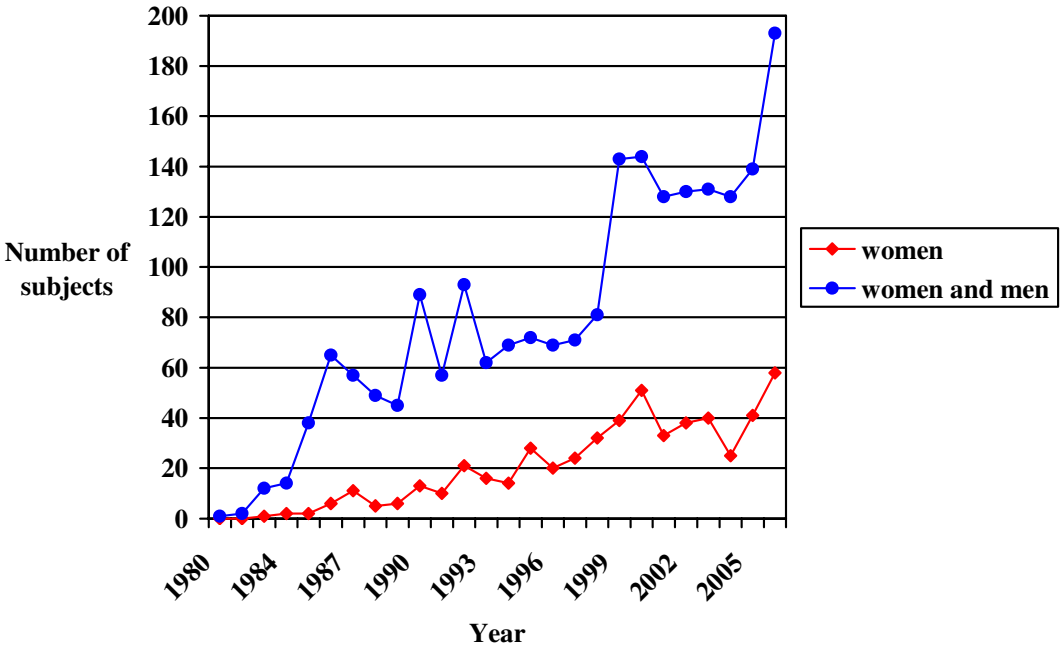


Figure 4. The annual number of women and men with new diagnosis of HIV infection between 1980 and 2006 in Finland.

Table 2. Prevalence of HIV infection in Finland and her neighbouring countries and regions. (Leinikki, 2007).

Region	Prevalence of HIV/100 000
Region of St Petersburg	668
St Petersburg	452
Kalingrad	402
Estonia	376
Murmansk	165
Latvia	144
Karelia	50
Finland	40
Lithuania	29
Sweden	27

1.3 Transmission

At the beginning of the HIV pandemic, homosexual activity and intravenous routes were considered the main routes of transmission. Nowadays, heterosexual transmission of HIV is the main mode of infection among adults globally, while mother-to-child transmission accounts for most of the HIV infection in children. HIV particles are present in blood serum, semen, vaginal fluid, breast milk and other body fluids such as parotid saliva and urine (Simon *et al*., 2006).

The risk of HIV transmission is high during primary infections and HI viral levels decrease after the primary infection (Cohen *et al.*, 2005). It has been estimated that an HIV-negative female partner has a 0.1–0.5% risk of acquiring HIV per act of unprotected intercourse with an HIV-infected man after his primary infection (De Vincenci 1994, Gray *et al*., 2003). Co-infections with other sexually transmitted diseases (STDs) increase shedding of HIV and the risk of HIV transmission to a level similar to that in the primary infection (Galvin *et al*., 2004). In addition, STDs increase susceptibility to HIV infection by causing genital ulcers or inflammation (Galvin *et al*., 2004).

Women are at a greater risk of heterosexual HIV acquisition (European Study Group, 1989, Johnson *et al*., 1989, Padian *et al*., 1991). The reason for this may be the larger mucosal surface area and the longer duration of exposure. The HI virus survives well in semen, which is alkaline, in comparison with the acidic vaginal environment; alkaline semen may have a buffering capacity and may prolong HIV survival in the vagina (Simonsen *et al*., 1990). In addition, women's intravaginal practices (i.e. wiping, cleansing, douching, or the insertion of substances into the vagina) have been linked to an increase in susceptibility to HIV infection (Myer, Kuhn *et al*., 2005). However, individual genital mucosal immunity may also reduce the chance of acquisition of HIV in some women. Persistent seronegativity has been found among highly exposed prostitutes with certain human leucocyte antigen types (Plummer *et al*., 1999).

Figure 5 shows the trends of new HIV infections in Finland according to mode of transmission. Sexual transmission is the predominant mode in Finland. In 1998–1999 there was an epidemic among intravenous drug abusers, resulting in139 new HIV infections (www.ktl.fi/ hiv). The distribution of free syringes and increased information on HIV infection given to drug-abusers diminished the epidemic.

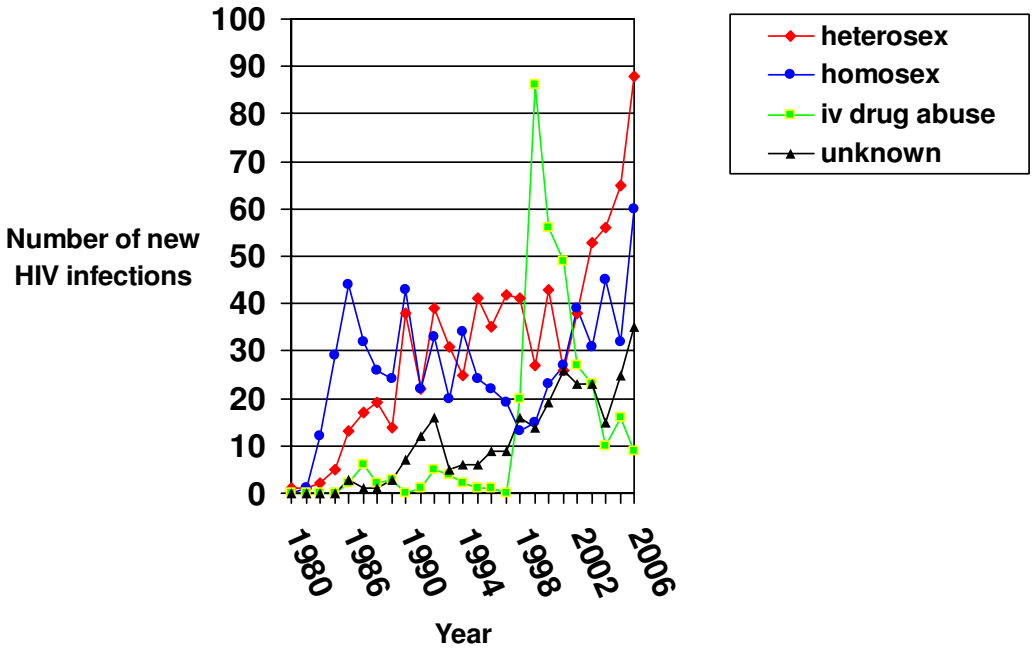


Figure 5. New HIV infections in Finland according to mode of transmission between 1980 and 2006. (www.ktl.fi).

2. HIV INFECTION DURING PREGNANCY

2.1 Diagnosis of HIV

Diagnosis of HIV is based on the detection of antibodies targeted to HIV. Use of an enzyme-linked immunosorbent assay (ELISA) followed by a confirmatory Western blot or immunoassay is the standard method. Both the sensitivity and specificity of this method are greater than 99% (Proffitt *et al*., 1993). Following primary infection, 50% of patients have a positive test result by 2 months and >95% are positive by 6 months (Horsburg *et al*., 1989). New rapid HIV tests also have good sensitivity and a specificity; 100% and 99%, respectively. The test result is available in 45–120 minutes compared with 28 hours for ELISA (Bulterys *et al*., 2004).

2.2 Screening of HIV during pregnancy

Different approaches exist for screening of HIV (Table 3). Under the opt-in approach women are provided with pre-HIV test counselling and must consent specifically to an HIV antibody test. Under the opt-out approach women are notified of the HIV test being included in a standard battery of prenatal tests and they may refuse testing. Under mandatory newborn HIV testing, newborns are tested for HIV if the mother's HIV test result is not available at the time of delivery, irrespective of maternal consent. The rates of HIV test acceptance vary in different countries and cultures, from 23% to 98% (Simpson *et al*., 1998, Fernandez *et al*., 2000, Joo *et al*., 2000, Martin-Hertz *et al*., 2006, Sherr *et al*., 2006). No clear association has been found between factors associated with HIV test acceptance or refusal (Carusi *et al*., 1998, Duffy *et al*., 1998). In a study among Nigerian women, almost all (96%) were willing to participate in HIV testing if it would help to prevent vertical transmission of HIV, but only a few would undergo the test if the result were to be shared with relatives (Ekanem *et al*., 2004). Good pre-test information has been found to increase acceptance of HIV testing. The addition of an HIV-focused nurse to clinical personnel increased the acceptance of HIV testing from 75% to 84% (Anderson *et al*., 2004). However, in a study in London hospitals, HIV test acceptance was found to be more likely if less than three minutes was spent discussing it (Sherr *et al*., 2006).

Screening for HIV in early pregnancy makes early diagnosis and early initiation of ARV medication possible in HIV-infected women and is, therefore, the most effective way to prevent vertical transmission of HIV. The city of Helsinki has offered opt-out HIV screening tests for pregnant women since 1986 and nationwide screening was started in 1998. Of all pregnant women, 99.8% accept the test in Finland (www.ktl.fi). In Sweden, voluntary HIV antibody testing was started in 1987 and the acceptance rate is also high, ranging from 90% to 99% (www.socialstyrelsen.se).

Table 3. Different strategies of screening for HIV

OPT-IN	OPT-OUT	MANDATORY
▪pre-test counselling ▪ informed consent needed for test	▪ information: HIV test included in standard protocol ▪ no informed consent needed ▪ possible to refuse the test	▪ newborn is tested if mother´s HIV status is not available ▪ with or without mother´s consent

2.3 The effects of pregnancy on HIV

Both HIV infection and pregnancy are characterized by increased immunosuppression, yet pregnancy does not accelerate the course of HIV infection (Burns *et al*., 1998, Weisser *et al*., 1998, Saada *et a*l., 2000,). In a large French study including women with a precise date of HIV seroconversion and performed before widespread use of HAART, women who delivered after HIV infection were compared with those who did not become pregnant while HIV-infected. The relative risk of progression from HIV infection to AIDS during pregnancy was 0.7 (Saada *et al*., 2000). Altered T-cell function (Rich *et al*., 1995) and a decline in mean CD4 cell count (Burns *et al*., 1996, Newell *et al*., 1997) have been reported during pregnancy. The clinical importance of these findings is, however, uncertain. Pregnancy itself does not seem to accelerate severe immunosuppression (CD4 count $< 0.2 \times 10^9$ cells/L), opportunistic infections or AIDS-related death (Buskin *et al*., 1998, Weisser *et al*., 1998, Saada *et al*., 2000).

2.4 The effects of HIV on pregnancy

Some studies have shown an association between HIV infection and miscarriage (Ryder *et al*., 1991, De Vincenzi *et al*., 1997, Brocklehurst *et al*., 1998,). In studies performed in developing countries greater frequencies of preterm birth, low birth weight, intrauterine growth restriction and stillbirth among the infants of HIV-infected women compared with HIV-negative women have been reported (Brocklehurst *et al*., 1998, Leroy *et al*., 1998). In industrialized countries these effects do not seem to exist (Bucceri *et al*., 1997, Brocklehurst *et al*., 1998). HIV infection does not increase the risk of malformations (Embree *et al*., 1989, Brocklehurst *et al*., 1998).

A role of the immune system as an aetiological factor of pre-eclampsia has been proposed (Dekker et al., 1998). Immunosuppression caused by HIV infection may inhibit immunological mechanisms and thus prevent the development of pre-eclampsia (Wimalasundera *et al*., 2002). In contrast, women on HAART may be at a higher risk of pre-eclampsia (Suy *et al*., 2006). However, at present, available studies on HIV and pre-eclampsia are small, retrospective or controversial. In a recent study performed in the Netherlands no change in the incidence of pre-eclampsia was found (Boer et al., 2007).

The risk of chorioamnionitis is higher among HIV-infected women (Gray *et al.*, 2007). At present, data on other obstetric complications such as hepatogestosis, hyperemesis or other infections is sparse.

2.5 Vertical transmission

2.5.1 Timing of transmission

Mother-to-infant transmission of HIV without any intervention occurs at rates of 14% to 50% (De Cock *et al*., 2000). Vertical transmission accounts for 90% of paediatric AIDS cases globally and almost all newly diagnosed HIV infections in children (Karon *et al*., 1996, Lindegren *et al*., 2000). Vertical transmission can occur at any time during pregnancy and delivery, but the probability of transmission is not equally distributed. In general, among women who do not breast-feed, one third of transmissions occur during gestation and the remaining two thirds during delivery (Kalish *et al*., 1997, Newell 1998). Some investigators suspect that the risk of vertical transmission is highest

during the last four weeks of pregnancy, and this could account for 50% of cases of vertical transmission (Kourtis *et al*., 2001). Figure 6 shows the estimated timing of vertical transmission according to a hypothetical cohort of 100 children born to untreated HIV-infected mothers (Kourtis *et al*., 2001)

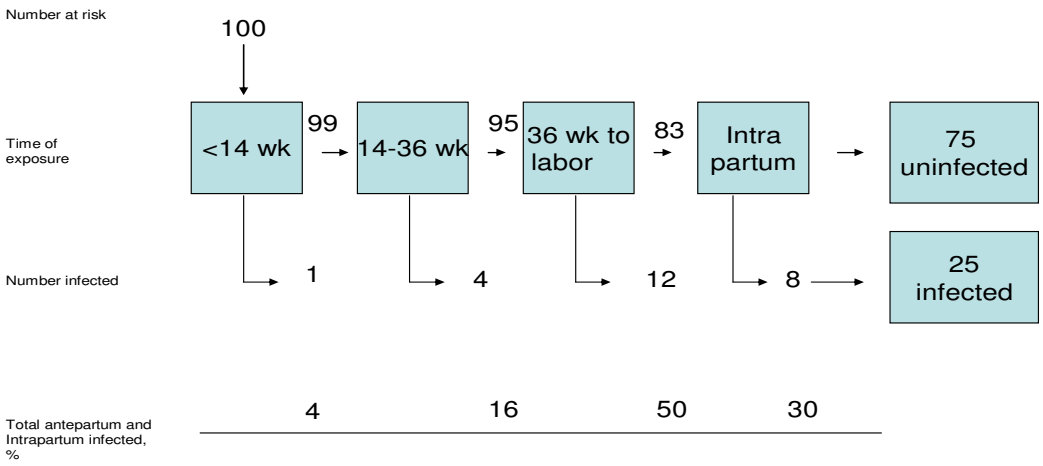


Figure 6. Estimated rates of vertical HIV transmission at different times of gestation and delivery in non-breast-feeding women. The model is based on a hypothetical cohort of 100 children born to HIV-infected women without any intervention (Kourtis *et al*., 2001).

2.5.2 Risk factors of vertical transmission

Maternal plasma HIV RNA load is the strongest independent predictor of vertical transmission (Mofenson *et al*., 1999). The risk is especially low among women with less than 1000 copies/mL. In the Women and Infants Transmission Study (WITS) the transmission risk was 0% among women with less than 1000 copies/mL, 16.6% among women with 1000–10 000 copies/mL and 40.6% among women with more than 100 000 copies/mL (Garcia *et al*., 1999). In a prospective transatlantic study the vertical transmission rate was 3.6% among women with an HIV RNA load of less than 1000 copies/mL (Ioannidis *et al*., 2001).

Among HIV-infected non-pregnant women with undetectable HIV RNA in their plasma, 25% display detectable HIV RNA in their vaginal lavage samples (Fiore *et al*., 2003). Thus, although the maternal plasma HIV RNA level is the strongest risk factor for vertical transmission, there is no level above which transmission always occurs or a level below which it is never seen.

A low maternal CD4 level has been associated with an increased risk of transmission (Mofenson *et al*., 1999). However, this association was noted before HIV RNA viral load measurements. Obstetric factors such as prolonged rupture (≥4 hours) of fetal membranes, placental abruption, use of fetal scalp electrodes, episiotomy and the presence of vaginal lacerations may increase the risk of vertical transmission (McGowan and Shah, 2000). The presence of chorioamnionitis and genital ulcers can further increase the risk (Lee *et al*., 1998, Mofenson *et al*., 1999, Wabwire-Mangen *et al*., 1999). The current risk of vertical transmission associated with amniocentesis and chorionic villus sampling during the HAART era is low, 3% (Somigliana *et al*., 2005). Preterm delivery is also associated with an increased risk of vertical transmission. Children born at 28 gestational weeks have a 60% risk of acquiring HIV infection among non-treated mothers; at the gestational age of 30 weeks the risk is 25% (European Collaborative Study, 1992).

Table 4. Risk factors associated with vertical transmission of HIV:

- high maternal HI viral load
- low CD4 lymphocyte level
- prolonged rupture of fetal membranes
- use of fetal scalp electrodes
- episiotomy
- vaginal lacerations, genital ulcers, chorioamnionitis
- preterm delivery
- vaginal delivery in unselected population
- non-use of ARV medication
- breast-feeding

2.5.3. Antiretroviral medication

In the absence of ARV medication the risk of vertical transmission is between 14% and 25% in developed countries and between 13% and 42% in developing countries where breast-feeding is common (Working group of mother-to-child transmission of HIV, 1995).

The efficacy of ARV medication in reducing vertical transmission was demonstrated first in 1994 (Connor *et al*., 1994). In this landmark study HIV-infected, non-breast-feeding women were given ZDV orally during pregnancy (started at a median of 26 weeks of gestation) and intravenously during labour. Thereafter the infants received ZDV for six weeks. Transmission rates were reduced by 68%, from 26% to 8%. Since then the results of many other studies have underlined the importance of ARV medication in prevention of vertical transmission (Table 5).

Since the mid-1990s, HAART has also been used during pregnancy. HAART is a combination of ≥ three types of antiretroviral medication of at least two different classes. Transmission rates of 1–2% have been published in patient series involving the use of HAART (McGovan and Shah, 2000, Bucceri *et al*., 2002). Shorter courses (from 34–36 weeks of gestation) of ARV medication are less effective than longer ones (from 24–26 weeks of gestation), although shorter courses also reduce transmission rates (Dabis *et al*., 1999, Wiktor *et al*., 1999, Lallemant *et al*., 2000). Compliance with the medication is essential in order to decrease levels of HI viral loads and the risk of vertical transmission. To achieve an undetectable HI viral load in 80% of patients, over 90% of the ARV medication must be used correctly (Paterson *et al*., 2000). Adherence to ARV medication during pregnancy has varied between 50% and 80% (Wilson *et al*., 2001, Ickovics *et al*., 2002). The use of HAART requires intensive monitoring by blood tests in order to prevent possible side-effects. Thus good financial resources are needed for appropriate follow-up.

In resource-poor countries single-dose ARV medication with NVP is a reasonable alternative. In Uganda NVP alone was 47% more effective than ZDV at reducing perinatal HIV transmission (Guay *et al*., 1999). However, NVP-associated resistance mutations have been detected in 15–67% of women following single-dose NVP (Cunningham *et al*., 2002, Eshleman *et al*., 2004, Shapiro *et al.*, 2006), limiting enthusiasm for this treatment. In a more recent study it was found that resistance occurred only when NVP-based ARV medication was initiated within 6 months after a single peripartum dose of NVP (Lockman *et al*., 2007).

No significant differences in the rates of congenital anomalies, low Apgar scores, low birth weight, or stillbirth between infants exposed to ARV medication and unexposed infants have been observed

(Tuomala *et al*., 2002). In a large prospective study it was found that there was an increased rate of premature birth associated with HAART treatment: adjusted odds ratios of 4.14 with protease inhibitors and 2.66 without protease inhibitors compared with no medication (European Collaborative Study, 2003). In the Swiss Neonatal HIV Study 33% of the women receiving HAART, but only 14% of women receiving no treatment, delivered prior to 37 weeks of gestation (Lorenzi *et al*., 1998). However, Tuomala *et al*. (2002) showed in a American multi-centre study (*n*=2123) that HIV infection *per se* did not increase the risk of premature delivery – 15% of women using combination therapy delivered prematurely, compared with 20% of women using no medication.

NRTI bind to mitochondrial DNA and can cause mitochondrial dysfunction manifested as myopathy, cardiomyopathy, neuropathy, lactic acidosis or fatty liver (Alimenti *et al*., 2003). The clinical impact of this dysfunction is unclear; in a review of ARV medication and neonatal outcome covering 20 000 children in the United States no deaths were found to be definitely related to mitochondrial toxicity (The Perinatal Safety Review Working Group, 2000).

Antiretroviral medication is generally relatively well tolerated during pregnancy. Anaemia is common among women using NRTIs; in a Swiss study 42% of women using ZDV and 3TC experienced anaemia (Lorenzi *et al*., 1998). The most common toxic effect of NNRTIs is rash. Nevirapine-related rash occurs in 17% of users and requires interruption of treatment in 5–6% of cases (Wood, 2002). PI has been linked to impaired glucose intolerance and an increased risk of gestational diabetes (Watts *et al*., 2004). Efavirenz is teratogenic and should not be used during pregnancy (www.aidsinfo.nih.gov).

Table 5. Trials in which the risk of vertical transmission has been assessed according to the antiretroviral agent used. * = statistically significant difference between different study arms.

Study	location	intervention	vertical transmission rate	breast-feeding
1. Trial with ZDV alone				
PACTG 076 (Connor *et al*., 1994) (*n*=363)	USA	ZDV from 14–34 weeks of gestation, intrapartum and to newborn for 6 weeks (=standard ZDV)	8%*	none
		Placebo	26%*	none
1A. Trials with ZDV started after 34 weeks of gestation				
Bangkok Collaborative Perinatal HIV Transmission Study (Shaffer *et al*., 1999) (*n*=392)	Thailand	ZDV from 36 weeks of gestation and during labour	9%*	none
		Placebo	19%*	none
Ivory Coast Trial (Wiktor *et al*., 1999) (*n*=230)	Africa	ZDV from 36 weeks of gestation and during labour	17%	100%
		Placebo	26%	100%
Perinatal HIV Prevention Trial (Lallemant *et al*., 2000) (*n*=1308)	Thailand	Standard ZDV	7%	none
		Standard ZDV, but ZDV for newborn only three days	5%	none
		ZDV started at 35 weeks of gestation, intrapartum, postnatally 6 weeks	9%	none
		ZDV started at 35 weeks of gestation intrapartum, postnatally 3 days	11%	none

Study	location	intervention	vertical transmission rate	breast-feeding
DITRAME (Dabis *et al*., 1999) (*n*=389)	Africa	ZDV from 36 weeks of gestation, during labour and to newborn postnatally	18%*	100%
		Placebo	28%*	100%
2. Trials with single-dose intrapartum NVP				
PACTG 316 (Dorenbaum *et al.,* 2002) (*n*=1270)	USA Europe Brazil Bahamas	Standard ZDV + placebo	2%	none
		Stardard ZDV+ NVP during labour and post-natally	1%	none
Perinatal HIV Prevention Trial (Lallemant *et al*., 2004) (*n*=1580)	Thailand	Standard ZDV	6%*	none
		Standard ZDV+ labour and postnatally	2%*	none
		Standard ZDV and NVP during labour	3%*	none
HIVNET 012 (Guay *et al*, 1999) (*n*=610)	Africa	NVP intrapartum and single-dose postnatally	12%*	99%
		ZDV intrapartum and postnatally for 7 days	20%*	99%
3. Trials with combination therapies and HAART				
PETRA (Saba *et al*., 2002) (*n*=1093)	Africa	A: ZDV + 3TC antepartum from 36 weeks of gestation, intapartum, postpartum for seven days to infant and mother	6%*	74%
		B: like Petra A, but no antepartum component	9%*	73%
		C: like Petra B, but no postpartum component	14%	76%
		Placebo	15%	74%

Study	location	intervention	vertical transmission rate	breast-feeding
PACTG 367 (Shapiro *et al*., 2000) (*n*=347)	USA	ZDV	7%*	none
		ZDV + 3TC	0%*	none
		HAART	0%*	none
		No medication/unknown	20%*	none
WITS (Cooper *et al*., 2000) (*n*=1201)	USA	ZDV	7%*	none
		HAART without PI	7%	none
		HAART with PI	0%*	none
Bucceri *et al*., 2002 (*n*=100)	Italy	HAART	0%*	none
Boer *et al*., 2007 (*n*=143)	Netherlands	HAART	0%*	none

Standard ZDV = ZDV started from 14–34 weeks of gestation, intrapartum and to newborn for 6 weeks

2.5.4. Vaginal versus Caesarean delivery

Since the majority of cases of HIV vertical transmission occur during or near the time of delivery, when fetal exposure to maternal body fluids is most likely, elective CS may reduce the risk. In a meta-analysis of 15 cohort studies, a 50% reduction in vertical transmission with elective CS compared with vaginal delivery was found (Andiman *et al*., 1999). The benefits of elective CS were additive with ZDV exposure; the likelihood of vertical transmission was reduced by 87% if both CS and ZDV were used compared with vaginal delivery and no ARV medication. However, the levels of plasma viral loads were not recorded. Hence the rate of elective CS has increased to 71–97% among HIV-infected women in some European countries (Thorne *et al*., 2001, Bucceri *et al*., 2002). European cohort study the effectiveness of elective CS in the HAART era was evaluated (European Collaborative Study, 2005). An odds ratio of 0.33 for vertical transmission with elective CS was found compared with vaginal delivery when adjusted for other factors, including ARV medication and viral load. The results suggest that offering elective CS to all HIV-infected women is

appropriate, especially for mothers with detectable viral loads. However, the value of elective CS in the management of women with low or undetectable HI viral loads has been questioned (Stringer *et al*., 1999, Ioannidis *et al*., 2001, Rowland *et al*., 2001). The protective effect of CS is less profound if the HI viral load is below 1000 copies/mL during the third trimester, because the transmission rate is as low as 1–2% (Ioannidis *et al*., 2001). Moreover, many (Watts *et al*., 2000, Read *et al*., 2001, Fiore *et al*., 2004), but not all (Parazzini *et al*., 1999) investigators have reported increased complication rates (e.g. postpartum fever, endometritis, wound infections, haemorrhagia, sepsis) associated with elective CS. The potential risks of elective CS may exceed the benefits among women with undetectable viral loads under ARV medication. European guidelines for management of HIV during pregnancy are that women should be given the option of elective CS (www.bhiva.org, Coll et al., 2002). However, US guidelines emphasize individualized obstetrics practices (www.aidsinfo.nih.gov). In Finland the decision on the mode of delivery is based on obstetric factors and HIV-RNA load during the third trimester.

2.5.5 Breast-feeding

Breast-feeding is responsible for most cases of post-partum HIV transmission (Kreiss, 1997). It has been estimated that the risk of transmission during breast-feeding is 14% for children of mothers with established HIV infection and 29% for children of mothers who acquire HIV infection after labour (Dunn *et al*., 1992). In an African trial among ARV-naive women it was found that breast-feeding was associated with a vertical transmission risk of 37% by the age of 24 months compared with 21% among children fed with formula (Nduati *et al*., 2000). However, the mortality rate was slightly higher in the formula-feeding group (24% *vs.* 20%). A similar result was also found in a more recent study among HIV-infected women (The Mashi study, 2006). Infants (n=1200) were randomized to 6 months of breast-feeding plus prophylactic ZDV, or formula feeding plus one month of ZDV. HIV-infection rates at seven months were 6% (formula-fed) *vs.* 9% (breast-fed), p=0.04. However, cumulative mortality at 7 months was significantly higher in the formula-fed group (9% *vs.* 5%, p=0.003). This study demonstrates the risk of formula feeding in developing countries. Mixed feeding (breast-feeding + formula feeding) is associated with even higher vertical transmission rates than breast-feeding or formula feeding alone (Coutsoudis, 2000). The explanation for this may be the infant's immune response to novel foods, which increases the amount of white blood cells in the gastrointestinal tract, providing additional targets for HIV infection (Coutsoudis, 2000).

3. GYNAECOLOGICAL PROBLEMS RELATED TO HIV INFECTION

3.1 Contraception

Approximately 70% of HIV-infected women are sexually active. However, the use of effective contraception is variable and unplanned pregnancies are frequent (De Vincenci *et al*., 1997, Desgrées-du-loû *et al*., 2002, Magalhaes *et al*., 2002). In a French SEROCO study, 20% of sexually active women used no contraception, 24% became pregnant, and 63% of these pregnancies resulted in abortion (De Vincenci *et al*., 1997). In the African DITRAME project, only 39% of HIV-infected women used contraceptives, 50% of all pregnancies were unplanned, and one third were terminated by abortion (Desgrées-du-loû *et al*., 2002). Lactational amenorrhoea is an important method of contraception in developing countries, but in order to reduce the risk of horizontal transmission of HIV and unplanned pregnancies, other methods, such as barrier methods, hormonal contraception and intrauterine devices are needed.

3.1.1 Barrier methods

Condoms represent the most effective method to prevent sexual transmission of HIV when correctly used. In the Cochrane database review of 4709 references, use of a condom in all instances of intercourse yielded an HIV incidence estimate of 1.14 per 100 person-years compared with 5.75 per 100 person-years among persons who never used condoms. The reduction in HIV seroconversion with condom use was approximately 80% (Cochrane Database 2002). The female condom has equal contraceptive efficacy as the male condom and potential HIV prevention efficacy (Mantell *et al*., 2006). The diaphragm, one of the oldest known contraceptive methods available for women, has a similar contraceptive efficacy to that of the male condom when used with a spermicide (Trussell, 2004), but the prevention of HIV transmission is minimal (van der Straten *et al*., 2005). Although the cervix constitutes the primary entry site for HIV (Moench *et al*., 2001), the cervical cap leaves the vaginal mucosa unprotected and the entry of HIV is possible through the vaginal epithelium (Farrar *et al.*, 1997).

Microbicides are chemical barriers that may be formulated as a gel, suppository film or incorporated into a sponge or intravaginal ring with slow release of the agent (Mantell *et al*., 2006). The spermicidal agent nonoxynol-9 was one of the first agents to be considered as a microbicide. In studies carried out *in vitro* nonoxynol-9 was active against HIV, but in clinical studies it was neither

safe nor effective (Hillier *et al*., 2005). Currently, approximately 60 candidate agents have been identified with *in vitro* activity against HIV, 18 of which have advanced to clinical testing, but they are not yet available for commercial use (D´Cruz *et al*., 2004).

Microbicides are divided into five classes according to the mode of action (McGowan, 2006):

1. Vaginal defence enhancers, which maintain an acidic pH in the vagina (Acidiform™, BufferGel™) or replace the absent lactobacilli (*Lactobacillus crispatus*). A rise in vaginal pH has proved to increase the susceptibility to HIV transmission (Myer, Denny *et al*., 2005).
2. Surfactants, in which the mechanism of action is disruption of cell membranes. However, they do not discriminate between mammalian and viral membranes, and this can lead to increased toxicity and decreased efficacy (e.g. nonoxynol-9, sodium layryl sulphate).
3. Viral entry and fusion inhibitors (e.g. Carraguard™, dextrin sulfate and cellulose sulfate)
4. Replication inhibitors, which contain ARV medication administered locally (e.g. tenofovir). The efficacy of tenofovir has ranged from 0.3–10% according to its gel concentrations (McGowan, 2006) and its safety and tolerability has been good (Mayer *et al*., 2006).
5. Combination products with more than one mechanism of action.

3.1.2 Hormonal contraception

In addition to condoms, many HIV-infected women choose to use hormonal contraception to minimize the risk of unplanned pregnancy. Dual contraception is generally recommended (Mitchell and Stephens, 2004). Hormonal contraceptives (combined oral contraceptives and progestin-only contraceptives) also have many health benefits such as cycle control, reduction in menorrhagia and dysmenorrhoea.

Based on international recommendations, no restrictions exist on the use of hormonal contraception for women at risk of HIV infection or for HIV-infected women (www.who.int/reproductive-health/stis/hc_hiv/nairobi_statement.html). Hormonal contraception used at the time of HIV

acquisition may affect the course of the HIV infection. In a study in which host factors were examined near the time of HIV acquisition, it was found that the use of injectable depot medroxyprogesterone acetate was associated with a higher HIV RNA load (Lavreys *et al*., 2004).

The circulating HIV load is the most important factor affecting cervicovaginal shedding of HIV RNA (Kovacs *et al*., 2001, Benki *et al*., 2004). However, one third of patients with a serum HIV RNA load below the detection limit have detectable HIV RNA in vaginal secretions (Kovacs *et al*., 2001, Fiore *et al*., 2003). In addition, cervicovaginal shedding of HIV was increased by use of NNRTI-based HAART compared with PI-based HAART among women with undetectable serum viral loads (Neely *et al*., 2007). In a prospective study of 17 HIV-infected women the level of cervical HIV shedding was lowest at the LH surge of the menstrual cycle. This nadir was followed by increasing levels of HIV cervical shedding towards menstrual bleeding (Benki *et al*., 2004). In studies involving non-human primates, progesterone increases the shedding of HIV and oestrogen decreases it (Marx *et al*., 1996). The use of hormonal contraception has been associated with modest increases in genital shedding of HIV in some (Mostad *et al*., 1997, Wang *et al.*, 2004), but not in all studies (Kovacs *et al*., 2001). The reason for this has been considered to be that the suppression of ovarian activity and low levels of circulating E2 result in thinning of the vaginal mucosa, reduced efficacy of the vaginal barrier, and increased shedding of HIV (Mingjia and Short, 2002). In a cross-sectional study in Kenya, cervical HIV shedding increased with increasing oestrogen dosage of the contraceptive pill (Mostad *et al*., 1997). The presence of cervical ectropion in association with combined oral contraceptives may also be a risk factor for acquisition of HIV (Moss *et al*., 1991, Howe *et al*., 1994). However, in a large multi-centre study (Uganda, Zimbabwe and Thailand) no association between hormonal contraception and HIV acquisition was found (Morrison *et al*., 2007).

Antiretroviral medication may reduce the efficacy of oral hormonal contraceptives by inducing the cytochrome P450 CYP 3A4 system of enzymes in hepatocytes of the liver and in enterocytes of the small intestine (Mitchell and Stephens, 2004). Progestogens and E2 are both substrates of this enzyme system. Table 6 lists antiretroviral drugs that may reduce the efficacy of oral hormonal contraceptives. Because of such enzyme induction, consideration should be focused on the oestrogen dose in contraceptive pills and a dose of 50 µg ethinyl oestradiol should be favoured, and the injection interval of progestogen reduced from 12 to 10 weeks, when many antiretroviral inducers are used (Elliman, 2000).

Table 6. Antiretroviral enzyme inducers that may reduce the efficacy of oral hormonal contraceptives.

Protein inhibitors
- Ritonavir
- Nelfinavir
- Lopinavir with ritonavir

Non-nucleoside reverse transcriptase inhibitors
- NVP
- Efavirenz

3.1.3 Intrauterine devices – copper and hormonal

Theoretical concerns about intrauterine device (IUD) use in women with HIV infection exist. These concerns consist of a possibly increased risk of pelvic infections and an increased risk of horizontal transmission of HIV as a result of increased volume and duration of menstrual bleeding and microtrauma to the penis caused by the IUD threads. In a study in Kenya 156 HIV-infected and 494 uninfected women were followed after insertion of a copper-IUD (Morrison *et al*., 2001). No differences in overall complications or infection-related complications were found. The incidence of pelvic inflammatory disease (PID) was low in both groups (2.0% in HIV-infected women and 0.4% in HIV-negative women). Cervical shedding of HIV after copper-IUD insertion was also studied. Among HIV-infected women no decrease in cervical shedding of HIV was found between baseline and four months of follow-up (Richardsson *et al*., 1999).

The levonorgestrel-releasing intrauterine system (LNG-IUS) is a highly effective long-term contraceptive method, with a Pearl index of 0–0.1 (Luukkainen and Toivonen, 1995). Unlike Cu-IUDs, the LNG-IUS reduces menstrual bleeding (Milsom *et al*., 1991). In addition, the LNG-IUS may reduce the risk of PID (Toivonen *et al*., 1991). Most women display normal follicular development and ovulation during LNG-IUS use (Nilsson *et al*., 1978, Barbosa *et al*., 1990). The LNG-IUS is also an alternative to surgical treatment of menorrhagia (Hurskainen *et al*., 2004). Thus, the LNG-IUS might be especially good for HIV-infected women, because surgical treatment of patients with advanced HIV infection is associated with an increased risk of operative and bleeding complications (Grubert *et al*., 1999). Moreover, use of the LNG-IUS in HIV-infected women suffering from bleeding disturbances may be especially advantageous. However, only one case-report of use of the LNG-IUS among HIV-infected women has been published (Cooling, 1999). Before widespread use of the LNG-IUS, it was considered as “grade 3” (theoretical or proved risks generally outweigh the advantages) among HIV-infected women (WHO medical

eligibility criteria, WHO, 1996). With increasing experience in use of the LNG-IUS, it has now been advocated for treatment of HIV-infected women (Guillebaud, 2004).

3.2 HIV and reproductive care

In developing countries mortality rates associated with HIV are high and reproduction is discouraged among HIV-infected women, although strong cultural pressures lead many to ignore this advice. By contrast, in developed countries the introduction of HAART has transformed HIV from a fatal to a chronic disease, and life expectancy approaches that of uninfected people. With HAART and with proper obstetric practices, vertical transmission rates have fallen from 40% to below 2% (European Collaborative Study, 2005). Therefore, it is no longer justifiable to deny fertility treatment to HIV-positive adults, the majority of whom are of reproductive age (Englert *et al*., 2001, FIGO Committee 2006). However, few centres in Europe offer IVF-treatment to HIV-infected women. No vertical transmission has been reported following IVF-treatment (Gilling-Smith *et al*., 2006).

HIV-infected women have relative subfertility (Glynn *et al*., 2000, Waters *et al*., 2007). Studies have shown increased tubal factor infertility (Frodsham *et al*., 2006) compared with uninfected women, whereas menstrual cycle disturbances are not increased (Harlow *et al*., 2000). Responses to ovarian stimulation are decreased among HIV-infected women, but ovum donation treatment has shown equal results compared with HIV-negative controls (Coll *et al*., 2006), reflecting the fact that HIV has an effect on ovarian function rather than endometrial function.
If a woman is HIV-positive and a man is HIV-negative, self-insemination and *in vitro* fertilization (IVF) are the means to eliminate the risk of horizontal transmission. In a European study on IVF among HIV-infected women there was a significant rise in serum HI viral load during ovulation induction. In addition, 60% of follicular fluid samples showed HI viral particles in women with an undetectable serum viral load (Gilling-Smith *et al*., 2006). This may have some effect on increasing the risk of vertical transmission.

In seminal plasma, HIV is present both as a free virus and as a cell-associated virus in the leucocytes and non-seminal cells. However, HIV does not seem to be able to infect the spermatozoa (Kim *et al*., 1999). Subsequently, if a man is HIV-positive and a woman is HIV-negative, semen washing decreases the risk of horizontal transmission. The risk of samples containing detectable

virus is 5–6%, because centrifugation fails to remove all seminal plasma and leucocytes (Gilling-Smith *et al*., 2006). Semen washing is also advisable for concordant couples in order to prevent a mutated, drug-resistant viral strain being transmitted.

The Centers for Reproductive Assistance To HIV couples in Europe (CREATE; United Kingdom, Italy, France, Belgium, Spain, Switzerland and Israel) collect data on semen washing and IVF. Between 1989 and 2003 the database consisted of 4989 semen washing cycles and more than 500 children born with no case of vertical transmission or seroconversion in the uninfected partner (Semprini *et al*., 2004).

3.3 Intraepithelial lesions of the uterine cervix

3.3.1 Epidemiology

The prevalence of precancerous lesions and cancer of the uterine cervix is increased among women infected with HIV (Serraino *et al*., 1999, Hawes *et al*., 2003). The prevalence of squamous intraepithelial lesions (SIL) and histologically verified CIN has been estimated to be as high as 20–40% (Wright, Ellerbrock *et a*l., 1994, Maimann *et al*., 1998, Ellerbrock *et al*., 2000). In the WIHS Study of 1713 HIV-infected women the overall prevalence of abnormal cervical cytology was 38%: 21% of the patients had ASCUS, 15% had LSIL, 2.3% had HSIL, and 0.2% had cancer of the uterine cervix (Massad *et al*., 1999). In the same study the prevalence of abnormal cytology among uninfected women was 16%. In the ALIVE study the prevalence of cervical abnormalities was 13% among HIV-infected women compared with 2.4% among HIV-negative women (Ahdieh *et al*., 2000). Ellerbrock *et al*. (2000) showed that HIV-positive women were 4-fold more likely than HIV-negative women to have CIN after a follow-up period of 36 months. In a Senegalese study HIV was strongly associated with the prevalence of cervical lesions, with LSILs or more serious abnormalities found in 17.2% of HIV-infected women compared with 4.0% of HIV-negative women (Hawes *et al*., 2003). In 1993, the US Centers for Disease Control (CDC) reported that cervical cancer was the most common cancer diagnosed among 16 784 women with AIDS. As a result, CDC added invasive cervical cancer (ICC) to the list of AIDS-defining illnesses of HIV (Table 1).

3.3.2 Risk factors of CIN

HPV

Sustained immunosuppression has been suspected to be responsible for the increased prevalence of multiple human papilloma virus (HPV) infections and for favouring oncogenic subtypes of HPV among HIV-infected women (Heard *et al*., 2000, Levi *et al*., 2004, Fontaine *et al*., 2005). Co-infection with HPV and HIV results in cell disruption and in dysregulation of the cellular and hormonal arms of local and systemic immunity, leading to the progression of cervical disease (Clark *et al*., 2002). In a study carried out at STD clinics in New York, HIV-infected women were significantly more likely to have cervical HPV infection at enrolment when compared with HIV-negative women (54% *vs*. 32%) and persistence of HPV infection and infection with high-risk types of HPV (types 16, 18) were more likely (61% *vs*. 23%) among HIV-infected women (Ellerbrock *et al*., 2000). In addition, in a WIHS study, HIV-infected women were more likely to be HPV-positive regardless of HPV type compared with HIV-negative women (Palefsky *et al*., 1999). In a Brazilian study of HIV-infected women, HPV DNA was detected in 98% of women and in 79% of cases there were multiple genotypes. Types 16 and 18 of HPV DNA were detected in 23% and 24% of women, respectively. The prevalence of high-risk genotypes increased with increasing abnormalities in Pap smears, but there was no significant association between the number of HPV genotypes and Pap smear classification (Levi *et al*., 2002).

Age

Young age is a well-characterized risk factor of SIL (Ho *et al*., 1998). Among HIV-infected women, young age has been recognized as a risk factor of SIL in some (Massad *et al*., 1999, Delmas *et al*., 2000, Ellerbrock *et al*., 2000), but not all studies (Wright, Ellerbrock *et al*., 1994).

CD4 count and HIV RNA load

A low CD4 lymphocyte level is a marker of the severity of immunosupression and together with a high HI viral load is associated with an increased risk of SIL. However, only the CD4 lymphocyte level has been identified as an independent risk factor of dysplasia (Davis *et al*., 2001). In a cohort of HIV-infected women with a known date of infection, women with a CD4 cell count of $< 0.2 \times 10^9$ cells/L had a twofold increase in prevalence of SIL compared with women with a CD4 cell count of $> 0.5 \times 10^9$ cells/L (Delmas *et al*., 2000). In a study by Cardillo *et al*. (2001) HIV-positive women with normal Pap smear results had significantly higher CD4 counts (0.378×10^9 cells/L)

than HIV-positive women with abnormal Pap smears (0.246×10^9 cells/L). The same difference was seen in HI viral loads (109 316 copies/L with Pap smear atypia *vs.* 41 602 copies/L with normal Pap smear findings). However, once the cervical lesion was established, disease progression was not affected by the CD4 lymphocyte level. The WIHS study showed no large absolute differences in the cumulative incidence of any type of SIL between HIV-infected women with a CD4 lymphocyte level of $> 0.5 \times 10^9$ cells/L compared with HIV-negative women (4% *vs.* 3%). However, HIV-infected women with a CD4 level of $< 0.2 \times 10^9$ cells/L showed a cumulative incidence of 9% as regards SIL at two years (Harris *et al*., 2005). In a Senegalese study, HSIL results in Pap smears were found in HIV-infected women only if the CD4 level was $< 0.5 \times 10^9$ cells/L and cancer of the cervix was detected only in women with CD4 cell counts of $< 0.3 \times 10^9$ cells/L (Hawes *et al*., 2003). In a French study of HPV DNA in cervical samples, a low HPV DNA level was a risk factor for cervical dysplasia only in severely immunosuppressed women (CD4 lymphocyte level $< 0.2 \times 10^9$ cells/L). A high HPV load was always associated with an increased risk of CIN, but a tenfold increase of risk was found in severely immunocompromised women (Heard *et al*., 2000). In conclusion, a low CD4 lymphocyte level, especially below 0.2×10^9 cells/L, is a clear risk factor of SIL.

Bacterial vaginosis (BV)

Bacterial vaginosis has been suggested to play a role in the development of CIN by increasing the risk of acquisition of HPV infection (Watts *et al*., 2005, Spinillo *et al*., 2006). In a study by Platz-Christiansen *et al.* (1994), CIN occurred in 5% of women with BV versus 1.4% of women without it. Bacterial vaginosis has also proven to be more prevalent and persistent among HIV-infected women, particularly among those who are severely immunocompromised (Jamieson *et al*., 2001). In addition, the detection of HIV in cervicovaginal secretions increases the risk of CIN (Spinillo *et al*., 2006).

Other risk factors

Other common risk factors of CIN in the general population are a history of abnormal cervical cytology, smoking, multiple sex partners, early sexual debut and parity. Among HIV-infected women, smoking seems to be the only independent risk factor of CIN (Heard *et al*., 1997). Smoking during HIV infection may alter the natural history of the infection and in this way increase the risk of CIN (Minkoff *et al*., 2004). Table 7 summarizes the risk factors of CIN.

Table 7

Risk factors of CIN among HIV-infected women

- HPV infection
  - HPV detected at the beginning of follow-up
  - high-risk HPV types
  - persistent HPV infection
- Young age
- Low CD4 lymphocyte level
- High HI viral load
- BV
- Smoking
- Genital shedding of HIV

3.3.3 Antiretroviral medication and CIN

The effects of HAART on morbidity and mortality and on immunological and virological markers of HIV disease progression are well documented (Palella *et al*., 1998, Hammer *et al*., 2005). However, a protective effect of ARV medication on the risk of CIN has not been satisfactorily established. HAART showed some potential effect on CIN in the WIHS study (*n*= 2059): women on HAART were 40% more likely to show regression and less likely to show progression of cervical lesions. No association was found between CD4 lymphocyte level and progression of cervical lesions among women on HAART treatment (Minkoff *et al*., 2001). However, the difference in regression rates between women with and without HAART was small (36% *vs.* 30%). In addition, in a study by Heard *et al*. (2002), HAART had a positive impact on regression of CIN, and this was associated with increasing CD4 cell counts. In other studies the effect of HAART on the prevalence of CIN has not been significant (Orlando *et al*., 1999, Lillo *et al*., 2001, Moore *et al*., 2002). In a study by Lillo *et al*. (2001), no effect on CIN was noted among women on HAART (*n*=163), not even among women who experienced the highest increase in CD4 lymphocyte levels after initiation of HAART.

In a prospective study among Italian HIV-infected women between 1981 and 1998 a trend of increasing incidence of ICC and other AIDS-defining diseases was observed during the period 1981–1995. This trend continued as regards ICC, whereas the incidence of other AIDS-defining diseases decreased (Dorrucci *et al*., 2001). The reason for this might be that decreasing mortality

from other AIDS-defining illnesses prolongs the exposure to HPV. HAART may not have had any effect in this study, since only a quarter of the patients used it.

In summary, current data on the effect of HAART on the natural history of CIN is controversial. Some types of ARV medication may have anti-HPV activity (Heard *et al*., 1998, Orlando *et al*., 1999), and HAART might be most beneficial among women in whom treatment is initiated at an earlier stage of the HIV infection (Palefsky, 2003).

3.3.4 Screening for CIN

The purpose of cervical screening is the early diagnosis of pre-malignant lesions, to prevent development of ICC. Screening programmes are also effective among HIV-infected women. Thus, ICC is uncommon among HIV-infected women participating in regular screening and prevention programmes in Europe and in the United States (Serraino *et al*., 2002, Massad *et al*., 2004). However, the prevalence of CIN is high worldwide, especially in developing countries, and ICC is the second most common cause of cancer death in women (Chirenje *et al*., 2005).

The value of Pap smears in screening for CIN among HIV-infected women is controversial. The validity of Pap smears was good (sensitivity 81%, specificity 87%) in a study carried out in New York (Wright, Ellerbrock *et al*., 1994). In this study Pap smears failed to reveal high grade CIN in only 0.8% of cases. An Italian study on the accuracy of Pap smears revealed similar results: sensitivity was 90% *vs*. 82% and specificity was 50% *vs.* 75% among HIV-positive and HIV-negative women, respectively (Branca *et al*., 2001). The results of the HIV Epidemiology Research (HER) study failed to support the use of routine colposcopy. In this study Pap smears were more likely to have false-negative results among HIV-infected women with low CD4 lymphocyte levels ($< 0.5 \times 10^9$ cells/L), but the abnormalities were of low-grade and 95% of these women revealed Pap smear abnormalities within one year after discordant results of Pap smears and colposcopy (Anderson *et al*., 2006). Some investigators have questioned the reliability of the Pap smear test in screening for CIN. Maiman *et al*. (1998) found a false-negative Pap smear rate of 18% compared with an expected rate of 4%. Although the risk of progression was low (9%) in a study by Robinson *et al*. (2003), follow-up by cytological testing was unreliable: Pap smears had a sensitivity of 19% in detecting high-grade lesions.

According to current guidelines HIV-seropositive women should have two Pap smears 6 months apart after the initial HIV diagnosis, followed by annual Pap smears if both are normal (Kaplan *et al*., 2002). However, it has been suggested that HIV-infected women with high levels of circulating CD4 lymphocytes and no detectable HPV in cervical specimens could be screened less rigorously (Harris *et al*., 2005). In contrast, screening by means of colposcopy and aggressive treatment of even low-grade lesions are preferred for immunocompromised HIV-infected women; CD4 lymphocyte levels of $< 0.2 \times 10^9$ cells/L and HPV DNA testing (types 16 & 18) may be useful risk indicators (Nappi *et al*., 2005).

An immunohistochemical test with monoclonal antibody $p16^{INK4a}$ seems to be capable of identifying HPV-positive cells and lesions of the cervix with an increased risk of progression to high-grade lesions (Queiroz *et al*., 2006). The level of proliferation marker Ki-67 is also higher in CIN lesions among HIV-infected women compared with HIV-negative women (Calore *et al*., 2001). These methods may be useful in the detection of CIN in the future.

3.3.5. Treatment of CIN

Several investigators have reported high treatment failure rates as regards CIN in HIV-positive women. A literature search of Medline and Cochrane libraries revealed a recurrence rate of CIN after the loop electrosurgical excision procedure (LEEP) ranging from 20 to 75% (Tebeu *et al*., 2006). However, mild changes (CIN1) have been shown to progress infrequently, even in women infected with HIV (Massad *et al*., 2004). In a study among French HIV-infected women, the annual rate of recurrence of CIN was 22.3%. Positive margins after LEEP and CD4 lymphocyte levels of $< 0.2 \times 10^9$ cells/L were risk factors for recurrence, whereas HAART exhibited a protective effect (Heard *et al*., 2005). In an American study also, margin involvement and low CD4 lymphocyte levels appeared to be risk factors, and HAART appeared to be protective as regards recurrence (Robinson *et al*., 2001). A low CD4 lymphocyte level has also been found to be a risk factor as regards recurrence of CIN in other studies (Wright, Koulos *et al*., 1994, Tate *et al*., 2002). LEEP treatment was not effective in eradication of CIN in the WIHS study; 45% of women with negative margins after LEEP treatment and negative endocervical results experienced recurrence (Holcomb *et al*., 1999). However, 42% of the patients did not use ARV medication. In the recently published WIHS and HERS studies, HIV-infected women undergoing LEEP for CIN were followed-up for 6

months using HPV testing and cytology. Recurrence was more likely among women treated for CIN 2 or CIN 3, those with CD4 counts < 0.2 x 10^9 cells/L, and those with detectable HPV after treatment (Massad *et al*., 2007). In a retrospective study of 68 HIV-infected women with matched HIV-negative controls, the first follow-up Pap smear following LEEP was normal in two thirds of HIV-negative patients, but in only one third of HIV-positive women. An association was found between the absence of recurrence and a viral response to ARV medication (Gilles *et al*., 2005).

AIMS OF THE STUDY

The effect of HIV infection among women has been studied widely in the world, but aspects of HIV infection among optimally managed HIV-positive Finnish women remain unclear. Although the LNG-IUS is a well-characterized contraceptive device, information on its use among HIV-infected women is very sparse. The aims of this study were to examine:

1. pregnancy outcome among HIV-infected women in the Helsinki metropolitan area between 1993 and 2003,

2. the safety and advantages or disadvantages of use of the LNG-IUS among HIV-infected women,

3. the risk factors and prognosis of squamous intraepithelial lesions and cervical intraepithelial neoplasia among HIV-infected women in Finland,

4. the optimal gynaecological follow-up of HIV-infected women

SUBJECTS

Table 8 summarizes the demographic characteristics of the subjects in Studies I–V.
In Study III the protocol was approved by the Ethics Committee of the Department of Obstetrics, University of Helsinki, and the Finnish National Agency for Medicines. The subjects gave informed consent prior to participation. Exclusion criteria were CD4 lymphocyte count below 0.35×10^9 cells/mL, untreated SIL in a Pap smear, acute PID or history of PID during the year preceding the study, current use of illicit drugs, active or chronic hepatitis, known Müllerian anomaly, use of oral contraceptives or progestins, and pregnancy.

Table 8. Demographics of the subjects

	Study I	Study II	Study III	Study IV	Study V
Number of subjects	45	12	6	108	153
Age (years) mean (range) at the beginning of follow-up	29 (18-39)	36(30-40)	35 (27-49)	31 (19-64)	31(20-50)
Duration of HIV infection (years), mean (range) at the beginning of follow-up	2.7 (1-10)	8.6 (5-12)	2.8 (0-9)	1.8 (0-13)	1.0 (0-13)
Duration of follow-up (years) mean (range)	1 (0.8-1.3)	1.0	3.8 (1-6)	4.4 (1-16)	5.6 (1-16)
Acquisition of HIV infection (%)					
heterosexual	78	100	83	79	76
drug abuse	9		17	12	14
unknown	13			9	10
Parity	1 (0-1)	1 (0-2)	1.5 (0-4)	1.5 (0-5)	1(0-5)
Hepatitis C (%)	8	0	67	17	20

METHODS

Management of the HIV-infected mothers was coordinated by a multidisciplinary team consisting of infectious disease specialists, paediatricians, obstetricians, nurses and social workers. The management of HIV infection was carried at the Department of Infectious Diseases. Visits to the Department of Obstetrics and Gynaecology were scheduled individually and the mean number of visits was 6.2 (range 2–13).

In all studies the hospital charts were reviewed as regards the route of HIV infection and duration of infection (defined as the time since the first positive HIV-antibody test result), timing and type of ARV medication used, country of origin, hepatitis C-seropositivity, and smoking. Of gynaecological parameters, gravidity, parity and contraceptive practices were noted.

The most recent (less than 6 months from the Pap smear carried out) CD4 lymphocyte count and the HI viral load were recorded in studies II, III, IV and V. In the pregnancy study, CD4 lymphocytes and HI viral load were measured at least every third month (Study I).

Pap smear results, as well as the results from the biopsy and LEEP specimens collected at the time of colposcopy were collected. The Pap smears were classified according to the Bethesda System (1991) as normal, atypical squamous cells of uncertain significance (ASCUS), atypical glandular cells of uncertain significance (AGUS), low-grade SIL (LSIL) or high-grade SIL (HSIL). Standard gynaecological pathology criteria and terminology were used to classify all intraepithelial lesions.

For prevalence analysis of SIL (Study IV), a single annual Pap smear from each woman was used. If more than one smear per year had been collected, the most recent smear was used. Because a large number of Pap smears were collected in 2000, 2001 and 2002, cytology results from these years were used to analyse the risk factors of SIL. The patients were divided into groups according to the CD4 lymphocyte count ($< \text{or} \geq 0.4 \times 10^9$/L), level of HI viral load (< 50 or ≥ 50 copies/mL), duration of HIV infection ($<$ or ≥ 5 years), use of ARV medication, presence of antibodies to HCV, and age ($<$ or ≥ 31 years).

For the analysis of natural history of cervical abnormalities (Study IV), all women with normal baseline Pap smear cytology and at least one follow-up Pap smear were included. When defining cumulative incidence, detection of any SIL was defined as an event.

For the analysis of CIN (Study V), the patients were divided into three groups according to the biopsy and/or LEEP results: no CIN, CIN1, and CIN2–3. The first group consisted of women having normal (*n*=19), ASCUS (*n*=53) or LSIL (*n*=30) Pap smear results, but normal biopsy and/or LEEP results. One case of cervical carcinoma was diagnosed during the follow-up period and this patient was included in group CIN2–3.
Pap smear results were recorded at the time of diagnosis of CIN, and at 6 and 12 months previously. The margins of the LEEP specimens were checked to assess the disappearance of CIN. Bacterial vaginosis was defined as the presence of clue cells in Pap smears.

In Study II, following recruitment to the study, breast and gynaecological examinations and vaginal ultrasonography (US) were performed. A cervicovaginal lavage specimen was collected by flushing the vagina and cervix repeatedly (i.e. 3–4 times) with 10 mL of physiological saline. An LNG-IUS (MIRENA®, Schering, Turku, Finland) was inserted approximately two months (range 1–4) after recruitment, between cycle days 1–7. A lavage specimen from the vagina and cervix, and a blood sample were collected prior to insertion of the LNG-IUS. Following insertion, correct location of the device was confirmed by vaginal US.
Gynaecological examination, vaginal US, collection of cervicovaginal lavage specimens, and blood sampling were performed at one week, 3 months, 6 months and 12 months following insertion of the LNG-IUS. Pap smears were collected at enrolment, and at 6 and 12 months. The subjects kept diaries of bleeding (requiring protection) and spotting (requiring no protection or use of panty liners only) during a 30-day period preceding insertion of the LNG-IUS, as well as at 5–6 and 11–12 months following insertion of the LNG-IUS.

Laboratory assays

Serum HIV RNA measurements: Between 1996 and 1999, the Amplicor HIV Monitor-test vs.1.0 (COBAS, Pleasenton, CA, USA), with a sensitivity of <400 copies/mL was used. From 1999 onwards the ultrasensitive COBAS Amplicor HIV Monitor-test vs. 1.5, with a sensitivity of <50 copies/mL was used.

Protein content of the lavage specimens was measured using Protein assay dye reagent (Bio-Rad Laboratoires Inc., Espoo, Finland). The HIV RNA load was measured using a COBAS TaqMan 48 HPS real time assay (CTM48 HPS; ROCHE Molecular Systems, Pleasenton, CA, USA), with a

sensitivity of <40 copies/mL. Quantification of HIV RNA in lavage specimens (Study II) was carried out using the CTM48 HPS system as follows: 0.5 mL of the lavage sample was passed through a High Pure System (HPS) column to isolate RNA. The obtained RNA was run in a CTM48 analyser together with internal controls. Control samples were created by using lavage specimens from HIV-negative women, with known amounts of HIV-positive plasma added. Protein content of the lavage specimens (0.5 mL) was measured thereafter, and the results are expressed as HIV RNA copies/mg of protein.

In Study II serum levels of oestradiol (E2) were measured by time-resolved fluoroimmunoassay, using commercial kits (Delfia, PerkinElmer Life Sciences, Turku, Finland). Levels of LNG were measured by radioimmunoassay (Suhonen *et al*., 1995). The detection limit of the LNG assay was 7 pg/mL (22 pmol/L). Serum levels of ferritin were measured in samples collected at the time of LNG-IUS insertion and at 12 months, using chemiluminescence immunoassays (Architect immunoanalyzer, Abbott Diagnostic Division, Abbott Park, IL, USA).

Data analysis

HIV and pregnancy (Study I)
Chi square tests were used to analyse the proportion of planned pregnancies and the use of ZDV infusion during delivery in different years. The Mann–Whitney *U* test was used to analyse the levels of CD4 lymphocytes.

HIV and SIL (Study IV)
The cumulative incidence of SIL was estimated according to the Kaplan–Meier method. The effect of the various risk factors on the cumulative risk of SIL was assessed using log-rank tests. Cox proportional hazard analysis was used for calculation of relative risks and for adjustment of relative risks. The χ^2 test and one-way analysis of variance (ANOVA) were used when appropriate. The statistical software used was SPSS version 12.0 from SPSS Inc. (Chicago, IL, USA), as well as Stat View software from the SAS Institute Inc. (Gary, NC, USA).

HIV and the LNG-IUS (Studies II and III)
The chi square test and paired sign *t* tests were used as appropriate. The statistical software used was StatView (SAS Institute Inc., Cary, NC, USA).

HIV and CIN (Study V)

The chi square test, paired sign *t* tests and Kruskall–Wallis tests were used as appropriate. Baseline measurements were analysed by fitting Cox´s proportional hazard model for each risk factor separately. For final models the Cox proportional hazard model was fitted. The baseline variables included age, duration of HIV infection, route of infection and number of births. In addition, the following variables were included as time-dependent covariates: smoking, use of alcohol, use of drugs, method of contraception, use of condoms, use of antiretroviral medication, HI viral load, CD4 lymphocyte result and BV result. The groups displaying CIN1 and CIN2–3 were combined for the uni- and multivariate analysis. The backward selection method was chosen, with a 10% significance level for removing an explanatory variable from the model. The sensitivity of the analysis was checked by means of a number of additional models including only selected variables. All results were computed by StatFinn Oy (Espoo, Finland), using SAS® version 9.1, SAS Institute Inc., Cary, NC, USA.

A two-tailed *p*-value lower than 0.05 was considered statistically significant.

RESULTS

Detailed results are given in the original publications and are therefore only briefly summarized here.

1. Pregnancy outcome among HIV-infected women (Study I)

Between 1993 and 2003 a total of 52 infants were born to 45 HIV-infected women at Helsinki University Central Hospital. The annual prevalence of HIV infection among the women delivering in the hospital district of Helsinki and Uusimaa increased from 0.6/10000 (95% CI 0–1.6) to 4.8/10000 (95% CI 1.4-8.2) between 1993 and 2002. HIV was diagnosed in the early pregnancy screening test in 40% of cases (18/45). Seventeen of the mothers received ARV medication prior to the pregnancy and in 34 cases (66%) the medication was started during the pregnancy. Among patients with HIV RNA load measurement performed during pregnancy, a good response (<1000 copies/mL) was obtained in 36/40 (90%); 24 (60%) of these women displayed undetectable HIV RNA loads during the third trimester. Nine patients had a complete set of samples of HI viral load and CD4 lymphocyte levels collected before pregnancy, during all three trimesters and after delivery. The levels of CD4 lymphocytes remained in the same range throughout pregnancy, and were somewhat lower in patients who had used ARV medication prior to pregnancy (Figure 7).

Combining all available CD4 lymphocyte levels analysed during the second trimester, the mean (range) levels were 0.39×10^9 cells/L (0.09–0.83 (n=14)) and 0.45×10^9 cells/L (0.25–0.95 (n=13)) in patients using ARV medication prior to pregnancy and in patients started on medication during pregnancy, respectively (NS). Figure 8 shows the distribution of ARV medication used during 1993–1998 and 1999–2003.

The majority (92%) of the infants was born at term, and their mean birth weight was 3350 g (SD±395 g). The CS rate was low, 25% (13/52). All newborns received ARV medication. Only one child was infected with HIV – the mother's HIV infection was diagnosed only after delivery.

There were no major pregnancy complications and the incidence of infectious complications was low, 12%. None of the mothers with infectious complications had a CD4 lymphocyte level below 0.2×10^9 cells/L. Eighteen mothers had recurrence of genital herpes simplex infection during

pregnancy. Their mean CD4 lymphocyte count was 0.50×10^9 cells/L (range 0.139–0.943) during the third trimester. Only three of them had low CD4 lymphocyte levels (i.e. $< 0.2 \times 10^9$ cells/L).

Table 9. Characteristics of the 52 deliveries among the 45 HIV-infected mothers

			number	**(%)**
Mode of delivery				
Vaginal delivery			39	(75)
Caesarean section			13	(25)
	elective			
		obstetric reason	3	
		HIV-related reason	4	
	emergency		6	
Beginning of labour				
contractions			32	(71)
rupture of membranes			13	(29)
Duration of pregnancy				
33–36 weeks			4	(8)
37–42 weeks			42	(80)
>42 weeks			6	(12)
CD4 lymphocyte ($\times 10^9$/L) level during the III trimester*				
<0.2			4	(9)
0.2–0.50			22	(48)
>0.50			20	(43)
Use of ZDV infusion during delivery				
Total			30	(58)
1993–1998			5/18	(28)
1999–2003			25/34	(74)

*Data available on 46 deliveries

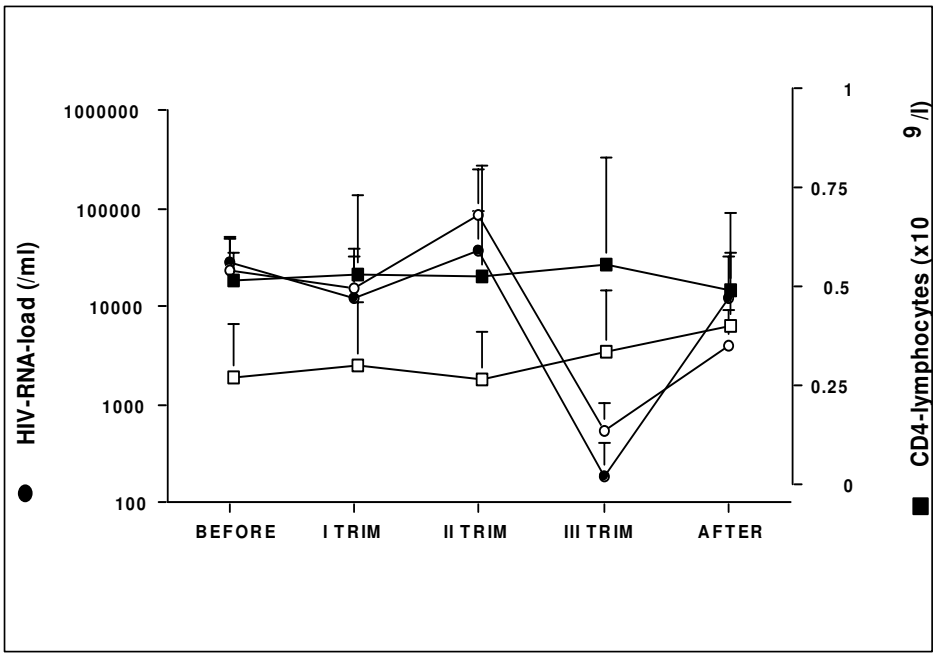


Figure 7. Circulating HIV RNA loads (circles) and the levels of CD4 lymphocytes (squares) (mean ± SD) of nine patients with samples collected before pregnancy, during each trimester and after delivery. Four patients received ARV medication prior to pregnancy (closed symbols) whereas in five the medication was started during the second trimester (open symbols). The data on HIV RNA load is presented on a semilogarithmic scale.

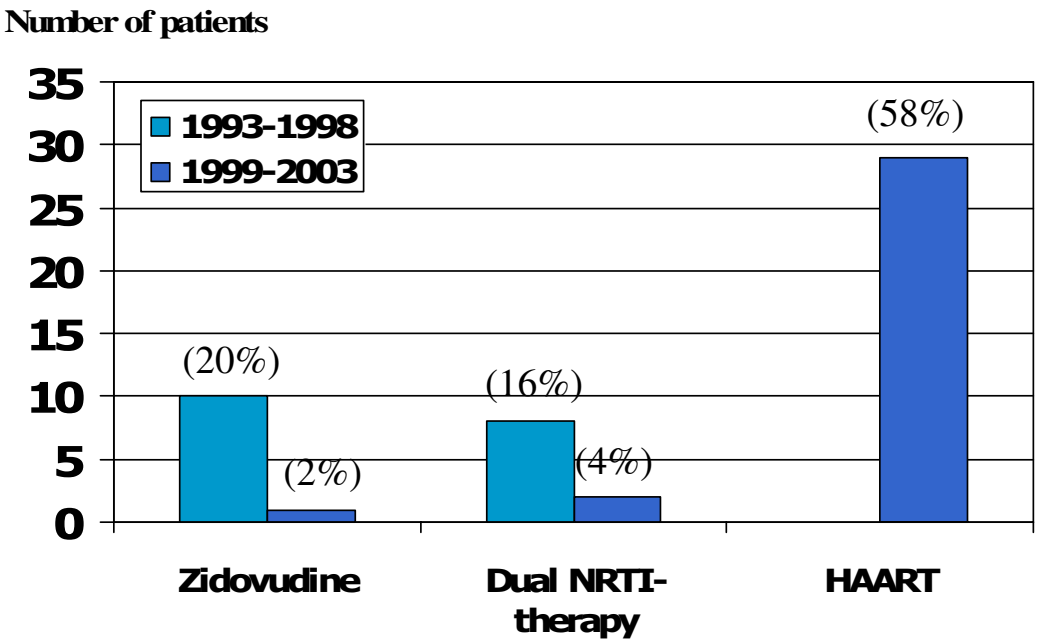


Figure 8. Numbers of patients receiving any type of ARV medication at the end of pregnancy during periods 1993–1998 and 1999–2003. The relative proportion of each treatment is shown in parentheses.

2. The LNG-IUS and HIV (Studies II and III)

In Study II use of the LNG-IUS was well tolerated and all subjects completed the 12 months of follow-up. The number (mean±SD) of days of bleeding decreased from 4.5 (±1.8) reported prior to insertion of the LNG-IUS to 2.4 (±2.6) (p=0.01) and 2.4 (±3.5) (p=0.10) at 6 and 12 months, respectively. The number (mean±SD) of days of spotting increased from 2.0 (±1.7) reported prior to insertion of the LNG-IUS to 4.5 (±3.1) at 6 months (p=0.02), thereafter decreasing to pre-treatment levels (2.7±2.2).

Reduction in bleeding was associated with slight increases in the levels of blood haemoglobin (135.0±8.3 *vs.* 138.6±14.1 g/L; p=n.s.) and serum ferritin (24.7±18.1 *vs.* 26.5±15.1 µg/L; p=n.s.) at one year of follow-up.

Serum levels of LNG were similar in the subjects with and without ARV medication. As expected, a slight decrease was noted in circulating levels of LNG over the study period (Figure 9, upper panel). Levels of E2 remained in the follicular phase range (i.e. >70 pmol/L) in all subjects throughout the study (Figure 9, lower panel).

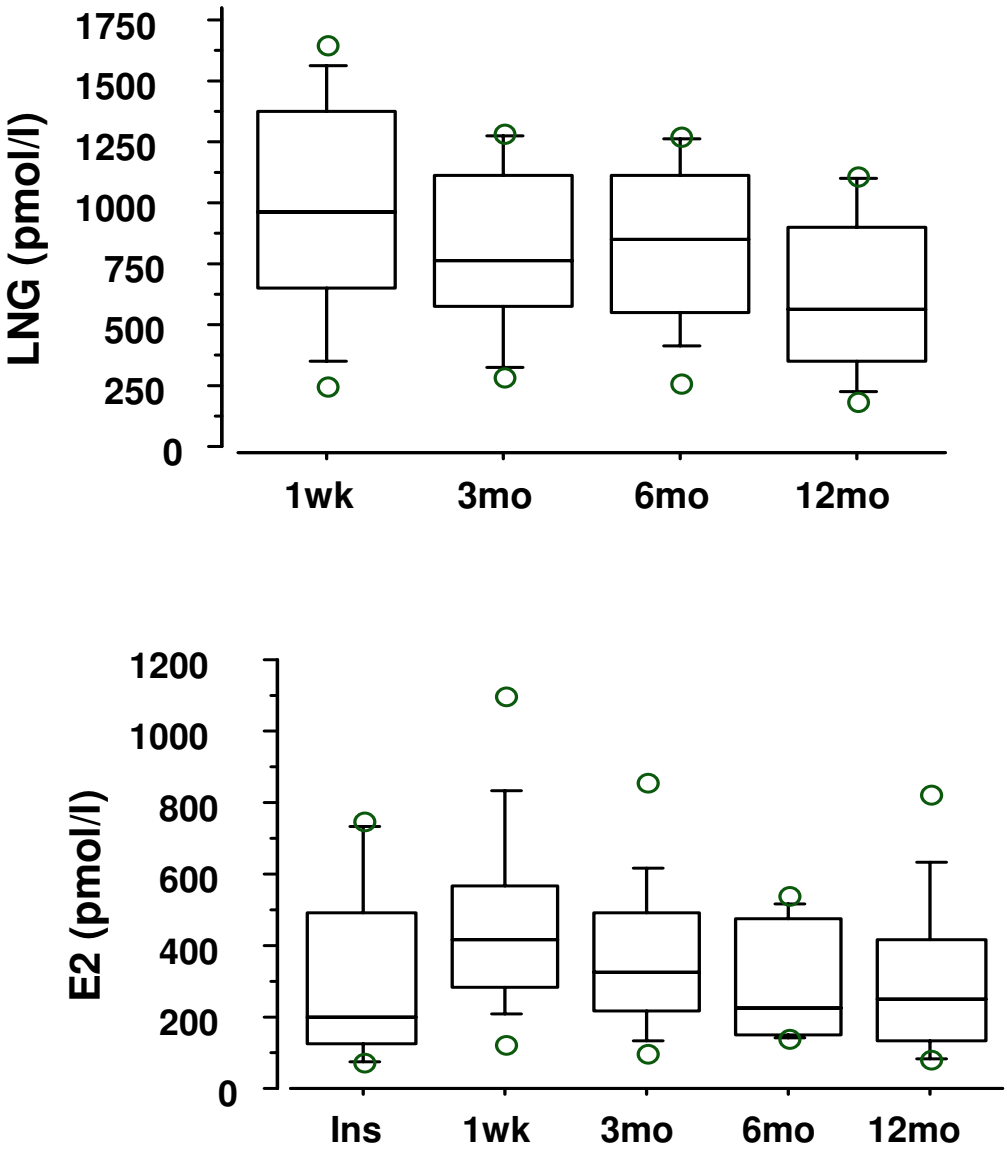


Figure 9. Circulating levels of LNG (upper panel) and E2 (lower panel) at insertion and at one week and 3, 6 and 12 months after LNG-IUS insertion.

The percentages of vaginal lavage samples with detectable HIV RNA in are shown in Figure 10. Results from subjects with and without ARV medication are shown separately. In women using ARV medication, HIV RNA was detectable in 10% of the lavage specimens collected both before and after insertion of the LNG-IUS. In samples with detectable HIV RNA, the viral load (mean±SD) was 638±822 copies/mg protein before, and 655±527 copies/mg protein after insertion of the LNG-IUS (*p*=n.s.). Among patients using ARV medication, the plasma level of HIV RNA was below the detection limit of the assay (40 copies/mL) in 49 of the 50 samples collected during the study period. Thus only one subject had a single measurable plasma HI viral load; all her vaginal lavage specimens were negative for HIV RNA. Of the two subjects who did not use ARV medication, the plasma levels of HIV RNA varied between 1350 and 23 600 copies/mL. In one, HIV RNA was detectable in the lavage specimens collected at recruitment, before insertion of the LNG-IUS and at 3 months (353, 795 and 120 copies/mg protein, respectively), whereas in the other, HIV RNA was detectable in the samples collected at 1 week and at 3 and 6 months (1693, 1238 and 10167 copies/mg protein, respectively). Levels of CD4 lymphocytes remained stable during the follow-up period. No subject had cytological changes in Pap smears consistent with SIL.

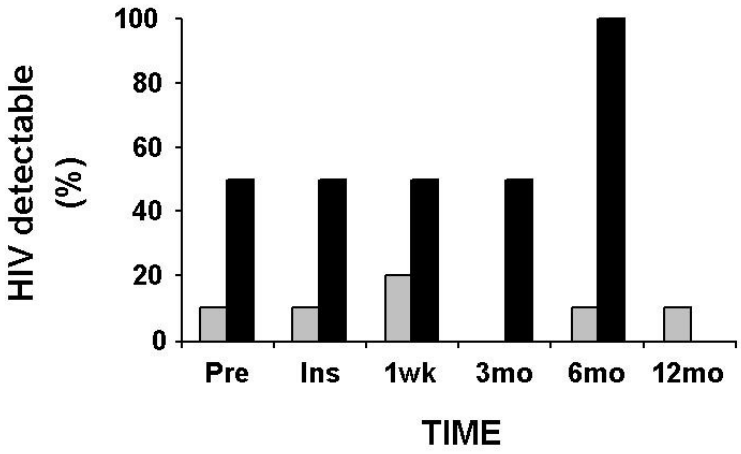


Figure 10. Percentage of vaginal lavage specimens with detectable HIV RNA. Samples collected from subjects using ARV medication (grey bars) and not using it (black bars) are shown separately.

In the retrospective study (Study III) on use of the LNG-IUS among HIV-infected women, the LNG-IUS was similarly well tolerated and no cases of unnoticed expulsion, pregnancy or PID were noted. The reported amount of menstrual bleeding was reduced in each subject. Blood levels of haemoglobin increased in each subject, the mean (±SD) rise being 12 (±10.6) g/L (range 1–31) (p=0.01). Levels of CD4 lymphocytes remained stable during the follow-up period. The distribution of Pap smear findings (no SIL *vs.* SIL) was unaltered during follow-up.

Figure 11 shows haemoglobin, CD4 lymphocyte as well as HIV RNA load values in the subject presenting with AIDS and menorrhagia necessitating blood transfusions associated with a large intramural leiomyoma. Insertion of the LNG-IUS resulted in rapid amenorrhoea, which was associated with increased blood haemoglobin levels. Concomitant use of HAART resulted in an unmeasurable HIV RNA load and an increase in circulating CD4 lymphocyte levels. High-grade SIL, diagnosed at 6 months, was treated by means of LEEP at 10 months, after which the Pap smear findings gradually normalized.

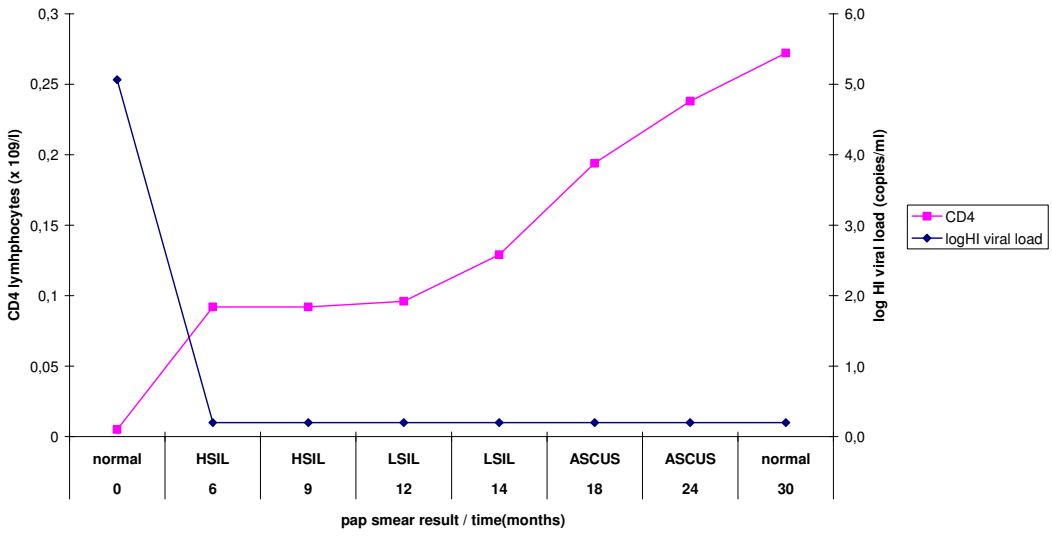


Figure 11. Changes in CD4 lymphocyte level, HI viral load and Pap smears in a single patient during the observation time of 30 months. HAART was started at the beginning of follow-up.

3. Prevalence and risk factors of SIL and CIN among HIV-infected women (Studies IV and V)

In the study concerning Pap smear findings (Study IV), 52% of all patients showed normal cytology in their Pap smears. The mean annual prevalence of LSIL was 15% and that of HSIL was 5%. Figure 12 shows the distribution of Pap smear results between 1995 and 2002. A reduced CD4 lymphocyte count ($< 0.4 \times 10^9$/L) was associated with an increased prevalence of SIL ($p<0.05$), (Figure 13).However, duration of HIV infection, use of ARV medication and HI viral load were not. Among the 55 women presenting initially with normal Pap smears, 18 developed LSIL and 6 developed HSIL during the follow-up period. Hence the cumulative risk of any type of SIL was 17% after one year (95% CI 7–27%) and it was 48% after five years (95% CI 33–63%). The risk of developing SIL was associated with young age ($p=0.04$) (Figure 14) and high initial HI viral load ($p=0.01$). However, the CD4 level, use of ARV medication, HCV co-infection and smoking were not associated with the development of SIL.

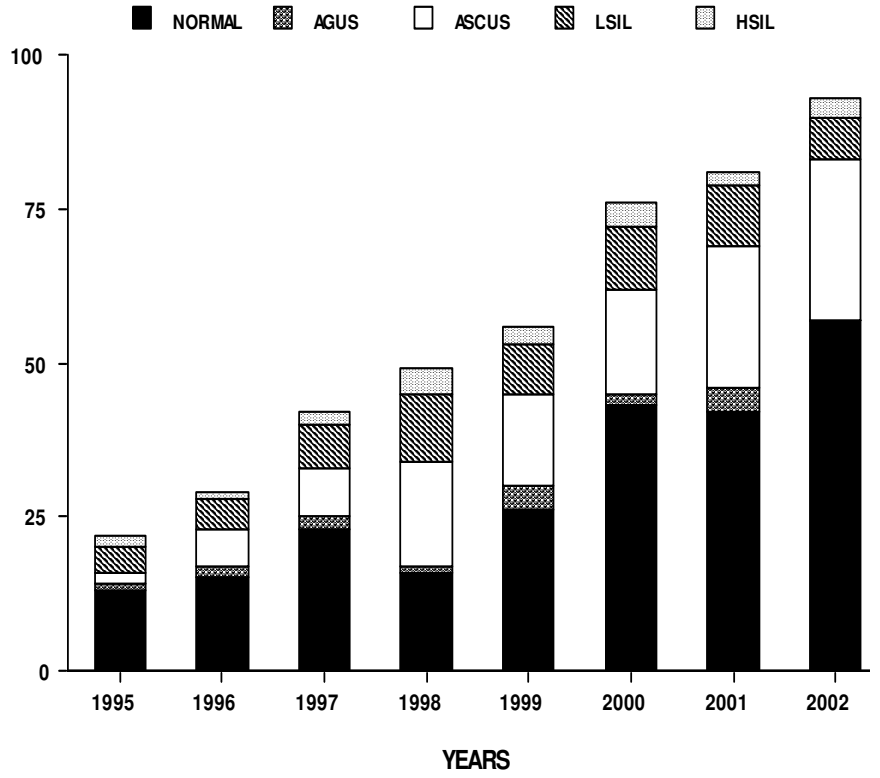


Figure 12. The Pap smear results of 108 HIV-infected women between 1995 and 2002.

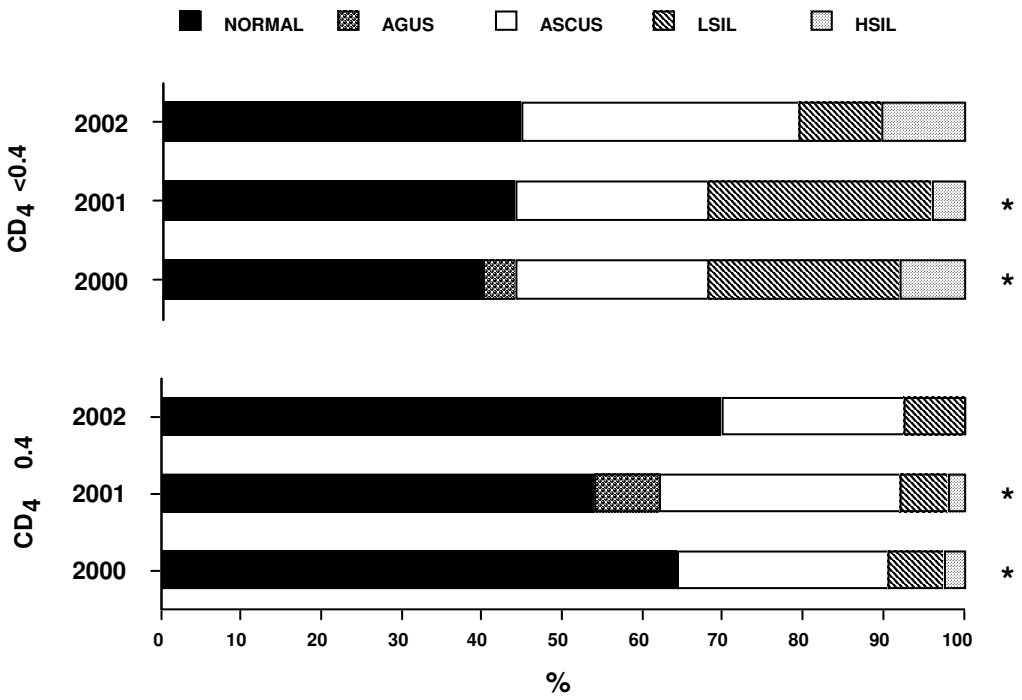


Figure 13. The influence of CD4 lymphocyte level (</≥ 0.4 × 10^9/L) on Pap smear findings in the samples collected in 2000, 2001 and 2002.

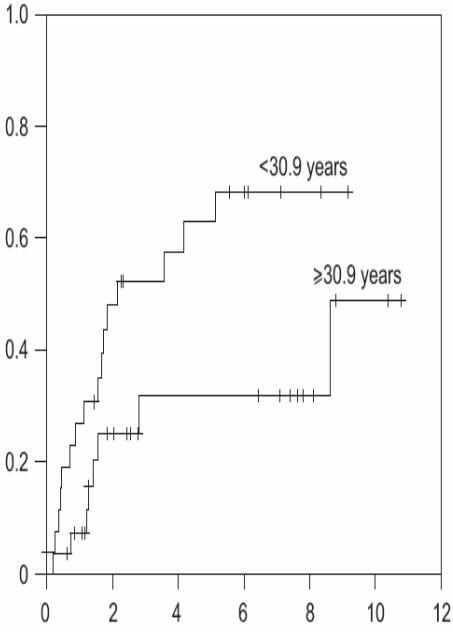


Figure 14. Cumulative risk of SIL among women presenting initially with normal Pap smears. The risk of SIL was increased among women younger than the median age (30.9 years).

In Study V, concerning histologically verified CIN, Pap smears were reliable in screening for CIN; 75% of patients with CIN had HSIL or LSIL in Pap smears taken at the time of dysplasia. The distribution of biopsy results and the corresponding Pap smear findings are shown in Table 10. During the follow-up period 51 subjects (33%) displayed CIN (16% CIN1 and 18% CIN 2-3), whereas 102 subjects had Pap smear results of normal cells, ASCUS, or signs of LSIL but no CIN. The incidence of CIN decreased from 12.7 to 3.5 (per 100 subjects) between 2000 and 2005 (p=0.07) (Figure 15). The risk of CIN was not associated with decreased levels of CD4 lymphocytes (Table 11). Table 12 shows univariate analysis of the effect of various baseline variables on the risk of CIN: only nulliparity (p=0.02) and bacterial vaginosis (p=0.04) were significantly associated with the risk of CIN. The hazard ratio of CIN among nulliparous women was 2.02 (95% CI 1.17 to 3.51) when compared with parous women. Each delivery lowered the risk of CIN by 30% (p=0.02). The hazard ratio of CIN was 1.85 (95% CI 1.04 to 3.28) among women who had BV at baseline. If a patient had BV in more than 25% of her Pap smear specimens, the risk of CIN was increased by 25.2% (hazard ratio 1.25; 95% CI 1.04 to 1.51) (p=0.02).

In multivariate analysis only nulliparity persisted as a significant risk factor of CIN (p=0.04). The results of the multivariate analysis were confirmed using a sensitivity model, in which both nulliparity (p<0.01) and BV (p<0.04) emerged as significant risk factors of CIN. Figure 16 shows the significantly increased cumulative risk of CIN as analysed by the Kaplan–Meier method, among nulliparous *vs.* parous women (*upper panel*) and among women who had *vs.* those who did not have BV at baseline (*lower panel*).

Cervical intraepithelial neoplasia was treated by means of LEEP (n=34). The recurrence rate was low; 7 subjects (14%) had a recurrence of CIN during follow-up. The nadir of CD4 lymphocytes was lower (p=0.04) and the HI viral load higher (p=0.03) among subjects with recurrence of CIN. Duration of HIV infection, use of ARV medication, and positive margins in LEEP specimens were indistinguishable among subjects with versus without recurrence of CIN.

Table 10. Pap smear findings in samples collected at the time of dysplasia.

	normal	ASCUS	LSIL	HSIL
CIN 1 (24)	2	5	13	4
CIN2-3 (27)	2	4	5	16

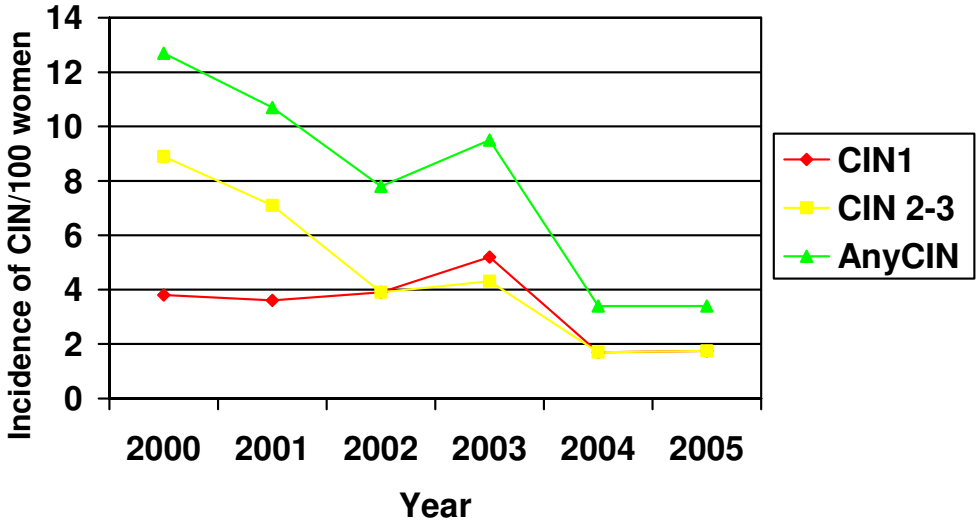


Figure 15. The decrease in the incidence of CIN/100 HIV-infected women between 2000 and 2005 approached statistical significance (p=0.07).

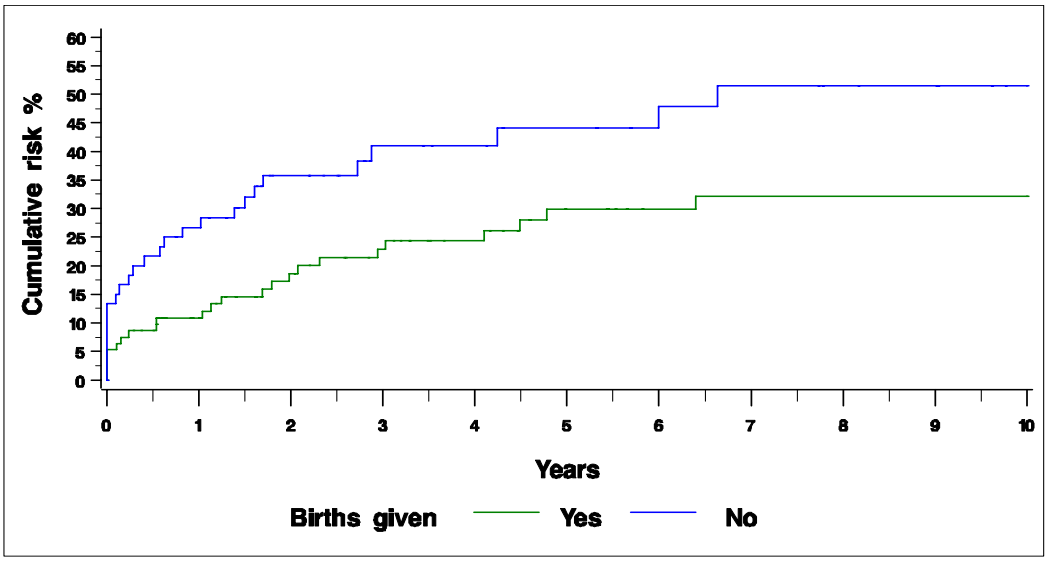
Table 11. Distribution of subjects with no CIN, CIN1 and CIN2–3 into different CD4 lymphocyte categories measured at the beginning of follow-up.

	No CIN (n=102)	CIN1 (n=24)	CIN 2–3 (n=27)
CD4 lymphocytes ($\times 10^9$/L)			
< 0.2	17 (17%)	5 (21%)	5 (19%)
0.2–0.5	43 (42%)	11 (46%)	12 (44%)
> 0.5	42 (41%)	8 (33%)	10 (37%)

There was no statistical difference in CD4 lymphocyte counts between CIN1 and no CIN (p=0.15) or between CIN2–3 and no CIN (p=0.14).

Table 12. CIN rates by different baseline variables among 153 patients

		No CIN	CIN (n=102)	Incidence/100 years (n=51)	p-value
Continent of origin					0.88
	Europe	64	34	7.84	
	Africa	17	12	9.95	
	Asia	15	5	6.06	
	Other	6	0	0	
Smoking					0.90
	yes	8	3	9.43	
	no	94	48	7.70	
Use of drugs					0.89
	yes	21	9	10.47	
	no	81	42	7.31	
C-hepatitis co-infection					0.67
	yes	20	10	11.0	
	no	82	41	7.19	
Parous					0.02
	no	33	27	12.35	
	yes	69	24	5.43	
Bacterial vaginosis					0.04
	no	83	33	6.45	
	yes	19	18	12.06	
Mode of HIV transmission					0.18
	Heterosexual	84	34	6.43	
	Injecting drug use	11	11	12.46	
	Other	7	6	13.64	
CD4 lymphocytes $\leq 0.2 \times 10^9$/L					0.90
	no	64	39	11.29	
	yes	14	8	14.58	
	data not available	24	4		
HI viral load below the detection limit of the assay					
	yes	19	11	11.37	0.77
	no	50	28	14.08	
	data not available	33	12		
Use of antiretroviral medication					0.59
	yes	41	24	7.81	
	no	61	27	7.37	



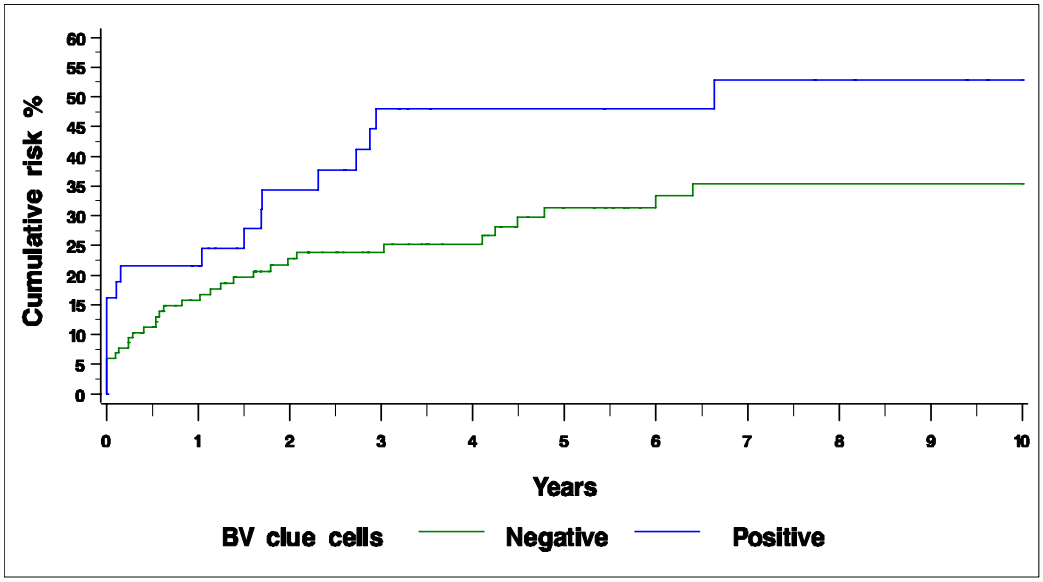


Figure 16. Cumulative risk of CIN among nulliparous *vs.* parous HIV-infected women (*upper panel*) and among women who displayed bacterial vaginosis *vs.* those who did not in Pap smear tests at the beginning of follow-up (*lower panel*). The cumulative risk of CIN was significantly increased among nulliparous women (p=0.02) and among women displaying BV (p=0.04).

DISCUSSION

We studied the pregnancy outcome, safety of LNG-IUS as well as prevalence, risk factors and prognosis of CIN among HIV-infected women in Finland. The main reasons for selecting these topics were firstly the fact that, in spite of national HIV prevention programmes, the prevalence of HIV-infection has not shown decreasing tendency in Finland (www.ktl.fi). Secondly, although the overall number of HIV-infected women in Finland is small (*n*=565 in July 2007), the HIV infection is spreading vigorously in our neighbouring countries (www.who.int). Thirdly, many studies on gynaecological aspects of HIV infection among women are derived from developing countries whose health care system differs from ours. Fourthly, the Department of Obstetrics and Gynaecology at the Helsinki University has arranged the follow-up of HIV-infected women systematically since 1986 and the compliance of patients to the care has been good. This has provided means for a long follow-up of patients in these studies.
The weakness of the study was a small number of patients compared to studies done in countries with a high prevalence of HIV infection. The retrospective nature of studies I, III, IV and V also has certain limitations. In addition, our study subjects were highly compliant. Thus, the present results are likely to be relevant only to HIV-infected women with access to high-quality health care services.

Pregnancy and HIV

Although pregnancy and HIV infection are both characterized by immunosuppression, pregnancy does not affaect the course of HIV infection (Saada *et al.,* 2000), and HIV infection does not increase the risk miscarriages and preterm births in developing counties (Brocklehurts *et al*., 1998). Without any intervention the risk of vertical transmission from mother to child is 14-50% (DeCock et al., 2000). However, the use of ARV together with proper obstetric practices and avoidance of breastfeeding have reduced the risk of vertical transmission to 1-2% (Bucceri *et al*., 2002, Lallemant *et al*., 2000).
Early diagnosis of HIV during pregnancy is essential in order to reduce vertical transmission. Thus, universal screening is important for identification of infected women. The national screening is available in Finland and also in Sweden. The acceptance rates are high: 99.8% (www.ktl.fi) and 90-99% (www.socialstyrelsen.se). In the present series HIV was diagnosed in screening test in early pregnancy in 40% of patients.

The incidence of HIV infection among pregnant women in Helsinki increased eightfold (from 0.6/10000 to 4.8/10000) between 1993 and 2003. The reasons for this increase are likely to be altered prognosis of HIV (Hammer *et al*., 2005) and improved pregnancy outcome (Watts *et al*., 2002). Also experience in the care of HIV-infected women increased in obstetrical units. This is supported, for example, by increased use of intrapartal ZDV (from 28 to 76%).

The role of CS among HIV-infected women is under debate. A meta-analysis of 15 prospective studies revealed that CS reduces the risk of vertical transmission independently of the effects of treatment with ZDV (Andiman *et al*., 1999). However, the HIV RNA load was not investigated in this study. According to a Cochrane review CS is an efficient intervention for the prevention of vertical transmission among HIV-infected women not on ARV, but the risk of post partum morbidity with CS is higher than that associated with vaginal delivery. Moreover, the risk of vertical transmission among women with undetectable or low HIV RNA load is unclear (Read *et al*., 2005). According to European consensus on management of HIV during pregnancy, HIV infected women should be given the option of delivering through CS (Coll *et al*., 2002). Therefore the rate of CS has climbed to 70-97% in many European countries. In Helsinki the CS was performed, if the third trimester HIV viral load exceeded 1000 copies/ml. The resultant CS rate (25%) was similar to the overall CS rate observed in our hospital during the same time. However, it is unclear, whether the limit should be below 500 copies/ml as in Netherlands (Boer *et al*., 2006) or even undetectable viral loads. Thus, more studies are needed to investigate the risk of vertical transmission among HIV-infected women with undetectable viral loads with and without ARV.

HIV and LNG-IUS

Condom is the most effective contraceptive method for prevention of horizontal transmission of HIV (Cochrane Database, 2002). To minimize the risk of unplanned pregnancy many HIV-infected women choose to use double contraception. Because the use of copper-releasing IUD increases the menstrual bleeding and the risk of PID, it has been viewed cautiously among HIV-infected women (www.fhi.org/en/RH/FAQs/contraHIV_fag.htm). In contrast LNG-IUS reduces bleeding and may reduce the risk of PID. This makes it an ideal contraceptive for HIV-infected women.

In both studies of the LNG-IUS use among HIV-infected women, the LNG-IUS was well tolerated and no cases of unnoticed expulsion, pregnancy or PID were noticed.

The ARV may alter the metabolism of contraceptive steroids and decrease the contraceptive power of steroids (Mitchell and Stephens, 2004). Among our patients using LNG-IUS, the levels of

circulating LNG were similar to those of healthy subjects (Suhonen *et al*., 1995). This suggests that the contraceptive effect of LNG-IUS is no different in women using ARV.

The suppression of endometrial epithelium is the main contraceptive mechanism of LNG-IUS (Luukkainen and Toivonen, 1995). Most women display normal follicular development and ovulation during the use of LNG-IUS. Similarly, in our study, the circulating levels of E2 remained in the follicular range in all subjects.

The key finding in this study was that the genital shedding of HIV was unaltered among these patients using LNG-IUS. Occasional cervicovaginal shedding of HIV was detected in 40% of the subjects on ARV. This is in line with the results of previous studies reporting 25-33% rates of vaginal shedding of HIV among women using ARV and having undetectable circulating HIV RNA load (Fiore *et al*., 2003).

Double contraception by means of barrier methods and LNG-IUS may thus be an ideal method for contraception among HIV-infected women. LNG-IUS decreases the menstrual bleeding and is likely to reduce exposure to infected blood. Moreover LNG-IUS appears – similarly as in uninfected women (Hurskainen *et al*., 2004) – valuable in controlling menorrhagia that would otherwise result in anemia and need for hysterectomy.

HIV and CIN

The prevalence and cumulative risk of cervical atypia is high among HIV-infected women (Wright, Ellerbrock *et al*., 1994, Delmas *et al*., 2000). In our study performed among systemically followed HIV-infected women, the mean annual prevalence of LSIL was 15% and that of HSIL 5%. During the follow-up the prevalence of histologically verified CIN was 34% (16% for CIN1 and 18% for CIN2-3). However, the incidence of CIN decreased between 2000 and 2005. The reasons for this are likely to be multifactorial: HIV-infected women may have fewer sex-partners and less exposure to HPV after the diagnosis of HIV, or it may also reflects the excellent compliance to the follow-up and treatment protocols of the patients.

In this study the sensitivity and specificity of Pap smears in detection of CIN were good and similar to those seen among healthy women in Helsinki area (Nieminen *et al*., 2004). Also positive margins after LEEP did not result in any additional risk of CIN recurrence and Pap smears were reliable in follow-up also among these patients.

Several studies have found a correlation between SIL and reduced CD4 lymphocyte count and high HI viral load (Hawes *et al*., 2003, Ellenbrock *et al*., 2000, Harris *et al*., 2005). In the present study

increased prevalence of SIL was associated with low CD4 lymphocyte level, whereas HI viral load or duration of HIV infection had little effect on the prevalence of CIN. Among patients with initially normal Pap smear finding high initial HI viral load - not a low CD4 lymphocyte level - and young age were risk factors for SIL.

However, among subjects with histologically verified CIN, low CD4 lymphocyte level did not appear to be a risk factor for CIN. Reduced CD4 lymphocyte levels were only associated with recurrent CIN. BV has been suggested to play a role in development on CIN among HIV-infected women (Watts *et al*., 2005). Similarly in our study BW was associated with increased prevalence of CIN. Also nulliparity increased the risk of CIN.

ARV has shown some potential effects on decreasing the prevalence of SIL or CIN in some studies (Minkoff *et al*., 2001, Heard *et al*., 2002). In other studies the protective effect of HAART has not been confirmed (Lillo *et al*., 2001, Moore *et al*., 2002). Similarly, HAART showed no protection for CIN or SIL in our study.

The clinical value of parameters such as CD4 lymphocyte level, HI viral load or use of HAART in gynecological follow-up of HIV-infected women appears to be limited. Regular follow-up by Pap smears and an easy access to colposcopy are efficient ways to prevent the development of cervical abnormalities and cancer of the cervix among HIV-infected women. Women with low CD4 lymphocyte levels, recurrent BV and poor compliance to follow-up should be followed up and treated more intensively. According to current guidelines HIV-seropositive women should have two Pap smears 6 months apart after the initial HIV diagnosis, followed by annual Pap smears if both are normal (Kapplan *et al*., 2002). This seems to be a reasonable way of follow-up also among systemically managed HIV-infected women with good compliance to the follow-up.

CONCLUSIONS

1. The combination of universal antenatal screening and a multidisciplinary management of HIV-infected pregnant women allows individualized treatment and prevents vertical transmission of HIV. It seems that the mode of delivery can be safely decided on the base of obstetric factors among patients with low HI viral loads.
2. The LNG-IUS appears to be a safe contraceptive method among HIV-infected women. ARV does not change the levels on levonorgestrel and the mechanisms of contraception of LNG-IUS. Importantly, the cervicovaginal shedding of HIV is unchanged among patients using LNG-IUS.
3. The prevalence of SIL and CIN is strikingly high also among systemically followed-up patients. Low CD4 lymphocyte level appears to be a risk factor for SIL and recurrent CIN. Use of ARV, duration of HIV infection or HI viral load has little effect on prevalence of SIL or CIN. However, BV and nulliparity are associated with CIN.
4. Pap smear is a reliable method in screening and diagnosis of CIN among HIV-infected women. The follow-up with 12-months intervals seems to be reasonable in the gynaecological follow-up of HIV-infected women. Among patients with low CD4 lymphocyte levels more intensive follow-up may be needed. Similarly women with poor compliance to the treatment protocols should be treated more vigorously.

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